This Page Is Inserted by IFW Operations and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents will not correct images, please do not report the images to the Image Problems Mailbox.

WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau



INTERNATIONAL APPLICATION PUBLISH	HED I	UNDER THE PATENT COOPERATI	ON TREATY (PCT)
(51) International Patent Classification ⁷ :		(11) International Publication Number:	WO 00/05375
C12N 15/12, C07K 14/705, G01N 33/50, A61K 38/17 // C07K 16/28	Al	(43) International Publication Date:	3 February 2000 (03.02.00)
(21) International Application Number: PCT/USG (22) International Filing Date: 22 July 1999 (2 (30) Priority Data: 60/093,843 22 July 1998 (22.07.98) (71) Applicant (for all designated States except US): DERBILT UNIVERSITY [US/US]; 305 Kirklan Nashville, TN 37240 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): HELLERQVIST, [US/US]; 9014 Carondelet Place, Brentwood, TN (US). FU, Changlin [CN/US]; 5568 Saddlewood Brentwood, TN 37027 (US). (74) Agents: ASHTON, Nina, M.; Cooley Godward LL El Camino Real, Five Palo Alto Square, Palo A 94306–2155 (US) et al.	VAI N 3702 od Lan	BR, BY, CA, CH, CN, CU, CZ GD, GE, GH, GM, HR, HU, I KP, KR, KZ, LC, LK, LR, LS, MN, MW, MX, NO, NZ, PL, P SK, SL, TJ, TM, TR, TT, UA, ZW, ARIPO patent (GH, GM, UG, ZW), Eurasian patent (AI RU, TJ, TM), European patent ES, FI, FR, GB, GR, IE, IT, L patent (BF, BJ, CF, CG, CI, CI NE, SN, TD, TG). Published With international search repore	Z. DE, DK, EE, ES, FI, GB, ID, IL, IN, IS, JP, KE, KG, LT, LU, LV, MD, MG, MK, T, RO, RU, SD, SE, SG, SI, UG, US, UZ, VN, YU, ZA, KE, LS, MW, SD, SL, SZ, M, AZ, BY, KG, KZ, MD, (AT, BE, CH, CY, DE, DK, U, MC, NL, PT, SE), OAPI M, GA, GN, GW, ML, MR,
(54) Title: GBS TOXIN RECEPTOR			
(57) Abstract			
A novel GBS toxin receptor, and methods for its polypeptides are provided as well as detection, screening polynucleotides and polypeptides.	prepara g, and	ation and use are provided. GBS toxin re therapeutic methods and pharmaceutical co	eceptor polynucleotides and ompositions involving such

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS -	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
ΑT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
ΑZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	1E	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Келуа	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	zw	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		ZIIIIDADWC
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		-
EĒ	Estonia	LR	Liberia	SG	Singapore		

GBS TOXIN RECEPTOR

INTRODUCTION

Technical Field

This invention provides compositions and methods relating to GBS toxin receptor polynucleotides and polypeptides. The invention relates to a receptor for a polysaccharide isolated from a bacterial source.

Background

10

15

20

25

Group B \(\text{\beta}\)-hemolytic Streptococci (GBS) are ubiquitous microorganisms.

GBS is not known to cause any harmful infections in humans except for very young babies. GBS pneumonia, also called "early-onset disease", is associated with high morbidity and mortality in newborn infants.

In a series of studies conducted by Dr. Carl G. Hellerqvist and his associates at the Vanderbilt University School of Medicine, Nashville, Tennessee, a polysaccharide GBS toxin was identified. This toxin was determined to be a major factor in the complications of GBS pneumonia, and was found to be useful as a therapeutic agent in combating tumors though inhibition of vascularization (U.S. Patent No. 5,010,062).

In addition, as described in U.S. Patent No. 5,858,991 and WO98/32453, GBS toxin facilitates wound healing in patients by minimizing scarring and accelerating healing, and reduces wound-related tumor progression.

WO98/32452 and WO98/32448 describe the use of GBS toxin as a therapeutic agent for treating patients with chronic inflammatory diseases, such as rheumatoid arthritis and psoriasis, and for enhancing repair of neural injury.

Prior to this invention, receptors for GBS toxin had not been identified. The inventors, believing receptors of GBS toxin to reside on cells in the developing vasculature of tissues undergoing angiogenesis in the conditions described above, embarked upon a series of experiments resulting in the present invention.

5

15

20

25

30

SUMMARY OF THE INVENTION

For the first time, novel receptors for group B β -hemolytic Streptococcus GBS toxin (GBS toxin receptor) have been identified. One aspect of the invention provides a \bar{p} olypeptide comprising a GBS toxin receptor or polypeptide fragment thereof. Preferred embodiments include mammalian GBS toxin receptors. Also provided is an antibody that recognizes GBS toxin receptor or a fragment thereof. The polypeptide of the invention can be used, *inter alia*, for the screening of compounds that can be used to treat or prevent conditions arising from pathologic or hypoxia-driven

angiogenesis or neovascularization, such as, for example, cancerous tumors, chronic inflammatory disease, scarring during wound healing, keloids, neural injury, and reperfusion injury.

Another aspect of the invention provides a polynucleotide encoding a GBS toxin receptor or a fragment thereof and a polynucleotide hybridizable to such polynucleotide. Preferred polynucleotides are at least 10 bases in length and comprise a nucleic acid sequence encoding, or are complementary to a nucleic acid sequence encoding, a mammalian GBS toxin receptor or a polypeptide fragment thereof.

A third aspect of the invention is a complex comprising a GBS toxin bound to a mammalian toxin receptor or fragment thereof. Also provided is a method of forming such complex. The method comprises contacting a GBS toxin with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that permit specific binding of the GBS toxin to the polypeptide, and allowing the complex to form.

Yet another aspect of the invention is a method for purifying a compound that binds a GBS toxin receptor. The method comprises providing a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that binds GBS toxin, contacting the polypeptide with a sample comprising the compound under conditions that allow specific binding of the compound to the polypeptide, and separating the bound compound from the remainder of the sample.

Another aspect of the invention is a method of determining the presence or absence of GBS toxin in a sample. The method comprises contacting the sample with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that binds GBS toxin, under conditions that allow specific binding of GBS toxin to the GBS toxin receptor, and determining whether specific binding of GBS toxin has

occurred. Presence of GBS toxin in a sample obtained from a neonate is indicative of early onset disease.

A sixth aspect of the invention is a method for detecting pathologic vasculature in a mammalian tissue. The method comprises detecting the presence of a GBS toxin receptor. The method can be used for detecting or monitoring a variety of medical conditions associated with angiogenesis or neovascularization, such as, for example, detecting metastasis of a cancerous tumor, or monitoring the margin of a tumor in a mammal undergoing a therapy for cancer.

Another aspect of the invention provides methods for the identification of drug candidates for the treatment of medical conditions characterized by pathologic and/or hypoxia-driven angiogenesis or neovascularization. One embodiment is a method for identifying a compound that specifically binds a mammalian GBS toxin receptor. The method comprises combining a test compound with a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that allow specific binding to occur, and detecting a complex formed between the test compound and the polypeptide. Another embodiment is a method for determining cytotoxicity of a test chimeric compound. The method comprises exposing a cell expressing a mammalian GBS toxin receptor, or fragment thereof that binds GBS toxin, to a test chimeric compound comprising a cytotoxic agent coupled to GBS toxin, and detecting signs of toxicity. Yet another embodiment is a method for identifying an inhibitor of a GBS toxin receptor by incubating test cells that express GBS toxin receptor, or a fragment thereof, in the presence and absence of a test compound and under conditions in which the cells incubated in the absence of the test compound can proliferate or migrate, and comparing the proliferation or migration of the test cells incubated in the presence and absence of the test compound, wherein less proliferation or migration in the presence of the test compound is indicative of the test compound being an inhibitor of the GBS toxin receptor. An inhibitor of endothelial cell proliferation or migration can be identified by the above method, wherein less proliferation or migration of test cells in the presence of the test compound is indicative of the test compound being an inhibitor of endothelial cell proliferation or migration. A therapeutic compound for the treatment or prevention of a medical condition characterized by pathologic angiogenesis or neovascularization can also be identified by the above method, wherein less proliferation or migration of test cells in the presence of the test compound is indicative of the test compound being a candidate

5

10

15

20

25

therapeutic compound for the treatment or prevention of the medical condition.

The invention also provides a method for identifying a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor. The method comprises simulating and selecting the most probable conformations of a mammalian GBS toxin receptor, designing a chemically modified analog that substantially mimics the energetically most probable three-dimensional structure of the polypeptide, chemically synthesizing the analog, and evaluating the bioactivity of the analog. Also provided is a method for identifying a compound which binds to a mammalian GBS toxin receptor. The method comprises simulating and selecting the most probable conformations of a mammalian GBS toxin receptor, deducing the most probable binding domains of the polypeptide, designing a compound that would form the energetically most probable complexes with the polypeptide, chemically synthesizing the compound, and evaluating the bioactivity of the compound.

Another aspect of the invention is a method for the prevention or treatment of neonatal onset disease in a human neonate by administering an inhibitor of binding of GBS toxin to a human GBS toxin receptor.

Yet another aspect of the invention is a method for inhibiting pathologic or hypoxia-driven endothelial cell proliferation or migration in a mammalian tissue. The method comprises specifically binding a molecule to a GBS toxin receptor present on the surface of at least one cell in the tissue, the molecule being selected from the group consisting of a compound that can evoke an inflammatory response when bound to a GBS toxin receptor in a mammal, a chimeric compound comprising a cytotoxic compound coupled to a compound that specifically binds the GBS toxin receptor, an inhibitor of GBS toxin receptor phosphorylation, and an inhibitor of GBS toxin receptor activity.

The invention also provides a GBS toxin receptor or fragment thereof, an inhibitor of a GBS toxin receptor, or an inhibitor of binding of a GBS toxin to a GBS toxin receptor, for use in a method of treatment of the human or animal body or for the manufacture of a medicament for the treatment of a medical condition characterized by pathologic or hypoxia-driven angiogenesis or neovascularization. Also provided is a chimeric compound comprising a cytotoxic agent coupled to a compound that binds GBS toxin receptor for use in a method of treatment of the human or animal body.

Also provided are pharmaceutical compositions comprising an inhibitor of a

10

15

20

25

GBS toxin receptor and/or a chimeric compound comprising a cytotoxic agent coupled to a compound that binds GBS toxin receptor, and a pharmaceutically acceptable carrier.

The invention also provides kits comprising a GBS toxin receptor or fragment
 and/or reagents for detecting the presence of a GBS toxin receptor or polypeptide
 fragment thereof or the presence of a polynucleotide encoding same.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts a process of rational drug design.

FIGS. 2A and 2B depict the results of immunohistochemical analysis of GBS toxin receptor expression in cancerous and normal human ovary tissue, respectively, using antibody Pab55 as described in Example 4.

FIGS. 3A and 3B depict the results of immunohistochemical analysis of GBS toxin receptor expression in cancerous and normal human ovary tissue, respectively, using antibody Pab57 as described in Example 4.

FIGS. 4A-4C depict the targeted delivery of a chimeric compound to GBS toxin receptor expressed in a cancerous tissue as described in Example 6.

DESCRIPTION OF SPECIFIC EMBODIMENTS

20 **DEFINITIONS**

Generally, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, and nucleic acid chemistry and hybridization described below are those well known and commonly employed in the art. Standard techniques are used for recombinant nucleic acid methods, polynucleotide synthesis, and microbial culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification steps supplied by manufacturers are typically performed according to the manufacturer's specifications. The techniques and procedures are generally performed according to conventional methods in the art and various general references (See generally, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring

25

Harbor, N.Y.) which are provided throughout this document. The nomenclature used herein and the laboratory procedures in analytical chemistry, organic synthetic chemistry, and pharmaceutical formulation described below are those well known and commonly employed in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical formulation and delivery, and treatment of patients. As employed throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

By "GBS toxin receptor" is meant a proteinaceous molecule capable of binding a toxin from Group B β-hemolytic *Streptococcus* bacteria (GBS toxin), such as, for example, CM101. A GBS toxin receptor is usually found in nature on the surface of a cell. Recombinant membrane bound and soluble GBS toxin receptors can be produced by laboratory techniques known in the art and described herein.

The term "isolated polynucleotide" referred to herein means a polynucleotide that has been subjected to manipulation, such that the isolated polynucleotide is no longer associated with the chromosome or cell that the polynucleotide is normally associated with in nature in the same manner as it is normally associated in nature. An example of an "isolated polynucleotide" is a polynucleotide of genomic, recombinant, or synthetic origin or some combination thereof.

The term "isolated protein" referred to herein means a protein that is no longer associated with the cell that the protein is normally associated with in nature in the same manner as it is normally associated in nature, such as (1) a protein free of at least some other proteins from the same source, (2) a protein expressed by a cell from a different species, (3) a protein that does not occur in nature, and (4) a protein produced from cDNA, recombinant RNA, or synthetic origin or some combination thereof.

The term "polypeptide" is used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein, fragments, and analogs are species of the polypeptide genus.

The term "naturally occurring" means found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) found in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

The term "operably linked" refers to a juxtaposition wherein the components

5

10

15

20

25

so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

The term "control sequence" refers to polynucleotide sequences which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is necessary for expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. The term includes single- and double-stranded forms of DNA.

The term "oligonucleotide" referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring and non-naturally occurring oligonucleotide linkages. An oligonucleotide is usually a polynucleotide 200 bases or fewer in length. Preferably oligonucleotides are minimally 10 to 60 bases in length and most preferably

15-35 bases in minimal length. Oligonucleotides are usually single-stranded, e.g. for probes; although oligonucleotides may be double-stranded, e.g. for use in the construction of a gene mutant. Oligonucleotides of the invention can be either sense or antisense oligonucleotides. The term "naturally occurring nucleotides" referred to herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoroaniladate, phosphoroamidate, and the like. An oligonucleotide can include a label for detection, if desired.

5

10

15

20

25

By "complementary" or "complement" is meant that wherever adenine appears in a first nucleic acid sequence, thymine or uracil is found in the "complementary" sequence and vice versa, and wherever guanine appears in a first nucleic acid sequence, cytosine is found in the "complementary" sequence and vice versa.

The term "sequence identity" describes the proportion of base matches between two nucleic acid sequences or the proportion of amino acid matches between two amino acid sequences, i.e. the degree of identity between two sequences. When sequence identity is expressed as a percentage, e.g., 50%, the percentage denotes the proportion of exact matches over the length of sequence from a GBS toxin receptor sequence that is compared to some other sequence. Various computer alignment programs can be used to determine sequence identity. In its simplest form, % identity is calculated by dividing the number of exact matches between two nucleic acid sequences or between two amino acid sequences by the total number of nucleotides or amino acids in the reference sequence. For example, if there are 300 matches between sequences 400 amino acids in length, the sequences have 75% identity. Uracil and thymine are considered identical when comparing a ribonucleic acid sequence with a deoxyribonucleic acid sequence.

As applied to polynucleotides, the term "substantial identity" means that two nucleic acid sequences when optimally aligned, such as by the program BLAST 20 (Altschul et al., J. Mol. Biol. 215:403-410 (1990)), share at least about 85%, preferably at least about 90% sequence identity and most preferably 95% or greater sequence identity. When using computer alignment programs, gaps (in either of the two sequences) are permitted to maximize matching; gap lengths of 15 bases or less 25 are usually used; 6 bases or less are preferred; 2 bases or less are most preferred. When using oligonucleotides as probes or in treatments, the sequence identity between the target nucleic acid and the oligonucleotide sequence is generally not less than 17 target base matches out of 20 possible oligonucleotide base pair matches (85%); preferably not less than 9 matches out of 10 possible base pair matches (90%), and most preferably not less than 19 matches out of 20 possible base pair matches 30 (95%).

5

10

Preferably, bases which are not identical nevertheless are part of a degenerate codon that encodes the same amino acid at that amino acid position. Alternatively, bases which are not identical preferably are part of a degenerate codon that encodes a conservative amino acid substitution for that amino acid position.

As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned by the BLAST computer program, share at least about 80 percent sequence identity, preferably at least about 86 percent sequence identity, more preferably at least about 95 percent sequence identity, even more preferably at least about 99 percent sequence identity up to having one amino acid difference, and most preferably share 100% identity. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine. alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatichydroxyl side chains is serine and threonine; a group of amino acids having amidecontaining side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acid substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamic-aspartic, and asparagine-glutamine.

The term "hybridizable under high stringency conditions" referred to herein means capable of specific binding under conditions whereby only nucleic acid sequences having a substantial identity of greater than 95% with respect to each other will hybridize. These conditions are known in the art and discussed herein.

The term "degenerate codon" means any of the nucleotide codon triplets encoding a desired amino acid according to the genetic code. Codons can be selected based upon known preferred codon usage in a host organism such as *E. coli*.

The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-

5

10

15

20

25

occurring sequence deduced, for example, from a full-length DNA sequence. Fragments typically are at least 3 amino acids long, preferably are 5-10 amino acids long, more preferably are 10-50 amino acids long, even more preferably are more than 50 amino acids long and comprise at least one extracellular domain of a GBS toxin receptor. Most preferred are fragments that comprise the entire extracellular domains of a GBS toxin receptor, and preferably also comprise portions of transmembrane and intracellular domains sufficient to maintain the polypeptide fragment in a functional stereochemical conformation on the surface of a cell, lipid membrane, liposome, micelle, or other lipophilic structure.

The term "immunologically reactive" means having antigenic properties or being capable of being specifically bound by an antibody that can specifically bind GBS toxin receptor. A substance has antigenic properties if it can generate monoclonal or polyclonal antibodies when administered to an animal under conditions known in the art to facilitate the production of antibodies that will recognize and bind a particular antigen.

A "heterologous polypeptide" is a polypeptide different from polypeptides normally produced by a particular cell. For example, a GBS toxin receptor polypeptide or fragment thereof that is produced recombinantly in a cell that does not normally produce such GBS toxin receptor polypeptide or fragment thereof, is a heterologous polypeptide. A second polypeptide joined to a GBS toxin receptor polypeptide or fragment thereof is also a heterologous polypeptide if it is not joined to a GBS toxin receptor polypeptide in nature.

As used herein, the terms "label" or "labeled" refers to incorporation of a detectable marker, e.g., by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes (e.g., ³H, ¹⁴C, ³⁵S, ¹²⁵I, ¹³¹I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, ß-galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some

10

15

20

25

embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

The term "compound" as used herein preferably refers to a peptidic, peptidomimetic, organic, or other chemical molecule and also refers to a nucleic acid molecule or chemical derivative thereof. The compound can interact with, or be, the polynucleotides or polypeptides of the invention.

The singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise.

The SEQ ID NOs of the nucleic acid and amino acid sequences described herein are summarized below in Table 1.

Table 1
Nucleic Acid and Amino Acid Sequences

SEQ ID NO:	Type of Sequence	Description
SEQ ID NO: 1	nucleic acid	Partial human GBS toxin receptor (HP55)
SEQ ID NO: 2	amino acid	Partial human GBS toxin receptor (HP55)
SEQ ID NO: 3	nucleic acid	Sheep GBS toxin receptor (SP55)
SEQ ID NO: 4	amino acid	Sheep GBS toxin receptor (SP55)
SEQ ID NO: 5	nucleic acid	Primer
SEQ ID NO: 6	nucleic acid	Primer
SEQ ID NO: 7	nucleic acid	Full-length human GBS toxin receptor (HP59)
SEQ ID NO: 8	amino acid	Full-length human GBS toxin receptor (HP59)
SEQ ID NO: 9	nucleic acid	Human/Sheep consensus GBS toxin receptor
		coding region
		(with base codes a, c, g, t, m, r, w, s, y, k)
SEQ ID NO: 10	amino acid	Human/Sheep consensus GBS toxin receptor
		coding region (translation of SEQ ID No: 9)
SEQ ID NO: 11	nucleic acid	Human/Sheep consensus GBS toxin receptor
	· · · · · · · · · · · · · · · · · · ·	coding region
		(with base codes a, c, g, t, n)
SEQ ID NO: 12	amino acid	Human/sheep consensus GBS toxin receptor
		coding region (translation of SEQ ID NO: 11)

The headings provided herein describe the general topic discussed and are not intended to be exclusive of information discussed in other sections. Frequently, information, methods, compositions, and other aspects may be applicable to more than one embodiment of the invention and can be so combined.

Introduction

20 GBS toxin binds to tissues undergoing pathologic, hypoxia-driven, and

embryologic angiogenesis or neovascularization. The inventors have identified at least two mammalian GBS toxin receptors, which are described herein. Examples 1 and 2 describe the cloning and characterization of some GBS toxin receptors. The inventors have classified GBS toxin receptor as an integral protein with seven transmembrane domains. The predicted segments are shown in Table 7. The protein 5 has several putative sites for phosphorylation by cAMP-dependent kinase, protein kinase C (PKC), and casein kinase II (CK2). Typically, such integral proteins, upon binding of a molecule (e.g., a ligand or an extracellular messenger), undergo a conformational change which facilitates phosphorylation at phosphorylation sites such as those discussed above. The phosphorylation of the protein at these sites may 10 trigger a signal transduction cascade, which often results in proliferation or other nuclear responses of the cells which have been exposed to the binding molecule. Angiogenesis or neovascularization involves proliferation and migration of endothelial cells. As discussed in greater detail in Examples 4 and 5, GBS toxin receptor expression is correlated with medical conditions involving pathologic, 15 hypoxia-driven, and embryogenic angiogenesis or neovascularization. GBS toxin receptor polypeptides can be used for a variety of purposes, including screening for compounds that can inhibit endothelial cell proliferation and/or migration mediated by GBS toxin receptor and screening for cytotoxic chimeric compounds that can bind to and destroy cells expressing GBS toxin receptor. GBS toxin receptor polynucleotides 20 can be used for a variety of purposes, including the design of antisense polynucleotides that can block translation of messenger RNA encoding GBS toxin receptor.

25 POLYNUCLEOTIDES

One aspect of the invention provides for isolated polynucleotides at least ten bases in length encoding or complementary to a nucleic acid sequence encoding a GBS toxin receptor or a fragment derived therefrom. Preferably, the GBS toxin receptor is a mammalian GBS toxin receptor, more preferably an ovine, bovine or feline GBS toxin receptor, and most preferably a human GBS toxin receptor. The isolated polynucleotides can be naturally occurring or non-naturally occurring. The isolated polynucleotides can comprise a DNA sequence or an RNA sequence in which every T is replaced with U. For purposes of determining percentage identity, T is considered equivalent to U. Preferably, the polynucleotides include alleles of an

ovine, bovine, feline or human GBS toxin receptor, and can include alleles of GBS toxin receptor of other mammals. These polynucleotides can be isolated using polynucleotides derived from SEQ ID NOs: 1, 3, 7, 9 and 11, as described further below.

Polynucleotides, oligonucleotides and fragments of the invention selectively 5 hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. The polynucleotides can be hybridizable under high stringency conditions to a nucleic acid molecule having a nucleic acid sequence comprising at least 20 contiguous polynucleotides, preferably at least 30 contiguous nucleotides of SEQ ID NO: 1 or 10 SEQ ID NO: 3, and even more prefereably to the nucleic acid sequence of SEQ ID NO: 1, 3, 7, 9 or 11 or the complement of SEQ ID NO: 1, 3, 7, 9 or 11. Such polynucleotides can be used for performing selective, high stringency hybridization and are particularly useful for performing amplification of nucleic acid by polymerase chain reaction (PCR) to determine the presence or absence of GBS toxin receptor in a 15 sample, for isolating a naturally occurring nucleic acid encoding a GBS toxin receptor (see Example 3), as antisense molecules for blocking translation of GBS toxin receptor mRNA. Particularly preferred are polynucleotides hybridizable under high stringency conditions to a nucleic acid molecule having a nucleic acid sequence 20 comprising the nucleic acid sequence of nucleotides 266 to 1870 of SEQ ID NO: 7 (the putative full length coding region of a human GBS toxin receptor, excluding the start codon), nucleotides 266 to 1870 of SEQ ID NO:7 (the putative full length coding region of a human GBS toxin receptor, including the start codon), nucleotides 61 to 1542 of SEQ ID NO:1 (the partial coding region of a human GBS toxin receptor, excluding the start codon), nucleotides 58 to 1542 of SEQ ID NO: 1 (the partial 25 coding region of a human GBS toxin receptor, including the start codon), nucleotides 87 to 1568 of SEQ ID NO: 3 (the coding region of a sheep GBS toxin receptor, excluding the start codon), nucleotides 84 to 1568 of SEQ ID NO:3 (the coding region of a sheep GBS toxin receptor, including the start codon), or a complementary nucleic acid sequence thereof.

The polynucleotides can have an identity to the nucleic acid sequence of a corresponding region of SEQ ID NO: 1, 3 or 7 or the complement of a corresponding region of SEQ ID NO: 1, 3 or 7 in the range of about 85% to 100%, preferably greater than about 87% identity, more preferably greater than about 95% identity, and most

preferably about 99% to 100% identity. Particularly preferred are polynucleotides comprising the nucleic acid sequence of nucleotides 266 to 1870 of SEQ ID NO: 7, or nucleotides 87 to 1568 of SEQ ID NO: 3, SEQ ID NO: 9, SEQ ID NO:11, or a complementary nucleic acid sequence thereof.

Preferably, the polynucleotides comprise a nucleic acid sequence encoding, or complementary to a nucleic acid sequence encoding, a polypeptide having an identity to the amino acid sequence of a fragment of a GBS toxin receptor in the range of about 85% to 100%, more preferably greater than 86% identity, even more preferably greater than 95% identity, and most preferably 99% to 100% identity. Preferably, the fragment binds GBS toxin. Preferred fragments comprise all or a portion of residues 1 to 495 of SEQ ID NO: 2 or all or a portion of residues 1 to 536 of SEQ ID NO: 8. Particularly preferred are polynucleotides comprising a nucleic acid sequence encoding a polypeptide having 100% identity to the amino acid sequence of residues 1 to 495 of SEQ ID NO: 4, residues 1 to 495 of SEQ ID NO: 2, or residues 1 to 536 of SEQ ID NO:8.

Polynucleotides encoding naturally occurring GBS toxin receptor can be isolated from various tissue sources and cell cultures from different species that produce such a receptor by the methods described herein, such as, for example, cells from tumor endothelium, synovial tissue in rheumatoid arthritis, or hypoxic tissue deprived of or restricted from blood flow, such as in reperfusion injury or wounded tissue. Such polynucleotides can be isolated by hybridization using probes or by polymerase chain reaction using oligonucleotides, as well as by implementing other molecular biology techniques known in the art. Such probes and oligonucleotides typically comprise various regions of the sequence of SEQ ID NO: 1, 3, 7, 9 or 11, preferably of SEQ ID NO: 1, 3, or 7, or encode various regions of the sequence of SEQ ID NO: 2, 4, 8,10 or 12, preferably of SEQ NO: 2, 4 or 8.

Polynucleotides useful for cloning genes encoding GBS toxin receptors of various organisms can be determined by comparing the amino acid sequences of homologous proteins. (see Table 4). For example, conserved regions can be targeted for the synthesis of oligonucleotides or degenerate oligonucleotides to be used as probes for hybridization or nucleic acid amplification, techniques discussed further below and in Example 3. Stringency can be varied to achieve selective hybridization conditions whereby nucleic acid sequences having less than 95% identity with respect to each other will hybridize. These conditions are known in the art and discussed

5

10

15

20

25

herein and examples are provided. Generally, the nucleic acid sequence identity between the polynucleotides, oligonucleotides, and fragments of the invention and a nucleic acid sequence of interest will be at least about 85%, and more typically with preferably increasing identities of at least about 90%, 95%, 99%, and 100%.

Polynucleotides can be used as probes under high stringency wash conditions 5 and with corresponding hybridization conditions, as known in the art. Small polynucleotides, for example, polynucleotides 200 bases or fewer in length, are often referred to in the art as oligonucleotides. Techniques for using polynucleotides as probes to detect the same or related nucleic acid sequences is well known in the art. See, for example, Sambrook et al, especially Chapter 11, the text of which is herein 10 incorporated by reference. Usually, probes can be made from polynucleotides that are 10 to 200 bases in length. Preferably probes are made from polynucleotides 10 to 60 nucleotides in length and most preferably 12 to 40 bases in length. Specific probes can be designed based on results obtained using nucleic acid homology computer programs such as FASTA, which uses the method of Pearson and Lipman (Proc. Natl. 15 Acad. Sci. USA 85:2444-2448 (1988)) and shows the degree of identity between compared sequences. The size of the probe is dependent upon the region of the gene to which it will be hybridized. The size of the probe increases as the degree of homology to undesirable nucleic acid sequences increases. A probe 10-50 nucleotides 20 in length can be used, preferably more than 50 nucleotides, even more preferably more than 100 nucleotides, and most preferably a probe made from the entire coding region of a GBS toxin receptor will be used. To decrease the number of false positives, preferably two probes are used to identify clones that bind to both probes under hybridization and wash conditions. Oligonucleotides can be synthesized on an Applied BioSystems oligonucleotide synthesizer according to specifications provided 25 by the manufacturer.

Typically, hybridization and washing conditions are performed at according to conventional hybridization procedures. Typical hybridization conditions for screening plaque lifts (Benton and Davis (1978) *Science* 196: 180) can be: 50% formamide, 5 x SSC (sodium chloride, sodium citrate) or SSPE (sodium chloride, sodium phosphate, EDTA), 1-5 x Denhardt's solution, 0.1-1% SDS, $100-200 \mu g$ sheared heterologous DNA or tRNA, 0-10% dextran sulfate, 1×10^5 to 1×10^7 cpm/ml of denatured probe with a specific activity of about 1×10^8 cpm/ μg , and

incubation at 42°C for about 6-36 hours. Prehybridization conditions are essentially identical except that probe is not included and incubation time is typically reduced. Washing conditions are typically 1-3 x SSC, 0.1-1% SDS, 42-70°C with change of wash solution at about 5-30 minutes. Cognate bacterial sequences, including allelic sequences, can be obtained in this manner. For high stringency hybridization conditions, various parameters can be altered to increase the stringency of hybridization, such as by increasing the temperature of incubation with the labeled probe. Preferably, for greater flexibility in experimental design, the probe can be hybridized at a lower temperature, such as, for example, room temperature and the stringency can then be modified by altering the salt concentration and temperature of the wash solutions. For high stringency a wash temperature of greater than or equal to 42°C can be used, such as, for example, 68°C, in a wash buffer having a salt concentration less than 3X SSC, such as, for example, 0.1X SSC. In some cases, TMACL can also be used, particularly for polynucleotides rich in G-C base pairs in order to decrease non-specific binding. A lower stringency wash can be used to hybridize polynucleotides with lower identities or polynucleotides that are less than 60 base pairs in length. For a low stringency wash, temperatures of less than or equal to 42° can be used in a wash buffer having a salt concentration of greater than or equal to 2X SSC.

The invention includes methods for amplification of target nucleic acids, such 20 as the polymerase chain reaction ("PCR") technique. The PCR technique can be applied to identify related sequences in the genomes of various organisms and to detect nucleotide sequences in suspected samples, using oligonucleotide primers spaced apart from each other and based on the genetic sequence set forth herein. The primers are complementary to opposite strands of a double-stranded DNA molecule 25 and are typically separated by from about 50 to 450 nucleotides or more (usually not more than 2000 nucleotides). This method entails preparing the specific oligonucleotide primers followed by repeated cycles of target DNA denaturation, primer binding, and extension with a DNA polymerase to obtain DNA fragments of the expected length based on the primer spacing. Extension products generated from one primer serve as additional target sequences for the other primer. The degree of amplification of a target sequence is controlled by the number of cycles that are performed and is theoretically calculated by the simple formula 2n where n is the number of cycles. Given that the average efficiency per cycle ranges from about 65%

30

5

10

to 85%, 25 cycles produce from 0.3 to 4.8 million copies of the target sequence. The PCR method is described in a number of publications, including Saiki et al., Science (1985) 230:1350-1354; Saiki et al., Nature (1986) 324:163-166; and Scharf et al., Science (1986) 233:1076-1078. Also see U.S. Patent Nos. 4,683,194; 4,683,195; and 4,683,202, the text of each patent is herein incorporated by reference. Additional methods for PCR amplification are described in: PCR Technology: Principles and Applications for DNA Amplification ed. HA Erlich, Freeman Press, New York, NY (1992); PCR Protocols: A Guide to Methods and Applications, eds. Innis, Gelfland, Snisky, and White, Academic Press, San Diego, CA (1990); Mattila et al. (1991)

Nucleic Acids Res. 19: 4967; Eckert, K.A. and Kunkel, T.A. (1991) PCR Methods and Applications 1: 17, and; PCR, eds. McPherson, Quirkes, and Taylor, IRL Press,

Oxford, all of which are incorporated herein by reference.

In yet another embodiment, an antisense polynucleotide can be administered to a mammal to treat or prevent a medical condition involving pathologic and/or 15 hypoxia-driven angiogenesis. The antisense oligonucleotides of the invention can be synthesized by any of the known chemical oligonucleotide synthesis methods. Such methods are generally described, for example, in Winnacker, From Genes to Clones: Introduction to Gene Technology. VCH Verlagsgesellschaft mbH (H., Ibelgaufts trans. 1987). Any of the known methods of oligonucleotide synthesis can be utilized 20 in preparing the instant antisense oligonucleotides. The antisense oligonucleotides are most advantageously prepared by utilizing any of the commercially available. automated nucleic acid synthesizers. The device utilized to prepare the oligonucleotides described herein, the Applied Biosystems 380B DNA Synthesizer, utilizes -cyanoethyl phosphoramidite chemistry. Antisense oligonucleotides 25 hybridizable with any portion of the mRNA transcript can be prepared by the oligonucleotide synthesis methods known to those skilled in the art. While any length oligonucleotide can be utilized in the practice of the invention, sequences shorter than 12 bases may be less specific in hybridizing to the target GBS toxin receptor mRNA. and may be more easily destroyed by enzymatic digestion. Hence, oligonucleotides having 12 or more nucleotides are preferred. Sequences longer than 18 to 21 30 nucleotides may be somewhat less effective in inhibiting GBS toxin receptor translation because of decreased uptake by the target cell. Thus, oligomers of 12-21 nucleotides are most preferred in the practice of the present invention, particularly oligomers of 12-18 nucleotides. Oligonucleotides complementary to and hybridizable

with any portion of the GBS toxin receptor mRNA transcript are, in principle, effective for inhibiting translation of the transcript, and capable of inducing the effects herein described. Translation is most effectively inhibited by blocking the mRNA at a sife at or near the initiation codon. Thus, oligonucleotides complementary to the 5' 5 region of the GBS toxin receptor mRNA transcript are preferred. Secondary or tertiary structure which might interfere with hybridization is minimal in this region. Moreover, sequences that are too distant in the 3' direction from the initiation site can be less effective in hybridizing the mRNA transcripts because of a "read-through" phenomenon whereby the ribosome is postulated to unravel the antisense/sense duplex to permit translation of the message. (see, e.g. Shakin, J. Biochemistry 261, 10 16018 (1986)). The antisense oligonucleotide is preferably directed to a site at or near the ATG initiation codon for protein synthesis. Oligonucleotides complementary to a portion of the GBS toxin receptor mRNA including the initiation codon are preferred. While antisense oligomers complementary to the 5' region of the GBS toxin receptor transcript are preferred, particularly the region including the initiation codon, it should 15 be appreciated that useful antisense oligomers are not limited to those complementary to the sequences found in the translated portion of the mRNA transcript, but also includes oligomers complementary to nucleotide sequences contained in, or extending into, the 5' and 3' untranslated regions. Antisense nucleotides or antisense expression constructs can find use to treat or prevent diseases associated with pathologic or 20 hypoxia-driven angiogenesis and neovascularization, as inappropriate expression of GBS toxin receptor results in hyperproliferation of endothelial cells.

In one embodiment, the polynucleotides of the invention can exist in linear form. In another embodiment, the polynucleotides can exist in circular form as part of a plasmid.

In yet another embodiment, a probe or PCR primer comprises a group of polynucleotide species containing different degenerate codons at various positions, which polynucleotides encode, or are complementary to sequences encoding, a GBS toxin receptor in whole or in part. Such polynucleotides can be useful for isolating nucleic acid sequences encoding polypeptides having at least about 85% identity to the amino acid sequence of sheep or human GBS toxin receptor, such as, for example, GBS toxin receptors of other organisms. Typically, such polynucleotides are synthesized chemically as described above by programming a synthesizer to incorporate a particular combination of nucleic acid residues at a certain position.

25

Typical designations are shown in Table 2.

Table 2

Base Codes

Symbol	<u>Meaning</u>
Α	A; adenine
C	C; cytosine
G	G; guanine
T	T; thymine
U	U; uracil
M	A or C
R	A or G
W	A or T/U
S	C or G
Y	C or T/U
K	G or T/U
V	A or C or G; not T/U
Н	A or C or T/U; not G
D	A or G or T/U; not C
В	C or G or T/U; not A
N	A or C or G or T/U

POLYPEPTIDES

Another aspect of the invention provides polypeptides comprising (1) the full length GBS toxin receptor protein or a naturally occurring allelic variant thereof, (2) fragments of at least 3 amino acids of the amino acid sequence of SEQ ID NO: 2, 4, 8, 10 or 12, and (3) a GBS toxin receptor protein, polypeptide, or polypeptide fragment 5 having an amino acid identity in the range of about 80% to 100% to the amino acid sequence of a corresponding region of SEQ ID NO: 2, 4 or 8. Preferred fragments of the amino acid sequence of SEQ ID NO: 2, 4, 8, 10 or 12, are at least 5, 6, 7, 8 or 9 amino acids in length and are immunologically reactive, i.e., immunogenic. More preferred are fragments at least 25 amino acids in length and fragments comprising 10 the amino acid sequence of residues 181 to 419 of SEQ ID NO: 2 or residues 1 to 240 of SEQ ID NO: 4. Most preferred are fragments that can bind GBS toxin. Preferably, the GBS toxin receptor protein, polypeptide, or polypeptide fragment has an amino acid identity to the amino acid sequence of a corresponding region of SEQ ID NO: 2, 4 or 8 of at least about 86%, more preferably at least about 95% identity, even more 15 preferably at least about 99% identity up to having one amino acid difference, and most preferably 100% identity. Preferred polypeptides have at least about 89% identity, more preferably at least about 95% identity, even more preferably at least about 99% identity up to having one amino acid difference, and most preferably 100% identity to the amino acid sequence of residues 181 to 419 of SEQ ID NO: 2, residues 20 1 to 495 of SEQ ID NO: 4. Preferably, a full length GBS toxin receptor protein comprises the amino acid sequence of residues 1 to 495 of SEQ ID NO: 2, residues 1 to 495 of SEQ ID NO: 4, or residues 1 to 536 of SEQ ID NO: 8, or an allelic variant thereof. The polypeptides of the invention can include amino acids in addition to the GBS toxin receptor protein, polypeptide, or polypeptide fragment. Such polypeptides 25 typically comprise a heterologous polypeptide joined to a second polypeptide derived, as described above, from a GBS toxin receptor. Preferably the additional amino acids are covalently linked to the amino-terminal or carboxy-terminal terminus of the GBS toxin receptor protein, polypeptide, or polypeptide fragment.

Fragments or analogs of GBS toxin receptor can be prepared by those of ordinary skill in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. For example, such functional domains include domains conferring the property of induction of an inflammatory response upon binding of GBS toxin to the GBS toxin receptor. GBS toxin mediates

the binding and opsonization by C3 of endothelial cells that express the GBS toxin receptor. Such domains can comprise the binding site for GBS toxin, in whole or in part, or domains otherwise essential for GBS toxin receptor structure and/or function. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known (Bowie et al. (1991) *Science* 253: 164). Computerized prediction methods, such as, for example, a hydropathy profile as provided by the "Soap" program in PC/GENE can be employed to identify putative structural and functional domains. Using the method of Klein, Kanehisa and DeLise, Biochim Biophys Acta (1985) 815:468-476, the inventors have classified a sheep GBS toxin receptor, SP55, as an integral protein with seven transmembrane segments predicted. Such a protein is also known colloquially in the art as a "7-spanner". The predicted segments are set forth below in Table 3.

Table 3

Predicted Transmembrane Domains of SP55

	Inner Bo	oundaries	Outer Bo	undaries	Segment	P:I odds*
No.	From	To	From	То	Sequence	
1	232	248	226	252	FFGIVGIIWFILWICLV (232-248 of SEQ ID No. 4)	2.589323E-05
2	369	385	365	389	LIGMIGPAIFLVAAGFI (369-385 of SEQ ID No. 4)	1.007311E-03
3	458	474	456	479	TVFCIAAAINVFGAIFF (458-474 of SEQ ID No. 4)	2.482542E-03
4	137	153	135	157	LLLGFGIFATAIFTLFT (137-153 of SEQ ID No. 4)	7.564906E-03
5	42	58	42	58	LAFLSFFGFFVLYSLRV (42-58 of SEQ ID No. 4)	8.236557E-02
6	328	344	328	345	GFLSAVPYLGCWLCMI L (328-344 of SEQ ID No. 4)	.1925022
7	390	406	390	407	SLAVAFLTISTTLGGFC (390-406 of SEQ ID No. 4)	.8064944

^{*} Relates hydrophobicity of integral sequence to the hydrophobicity of the peripheral sequence. An integral sequence with a higher hydrophobicity number is more likely to be part of a transmembrane domain.

A computerized alignment of the amino acid sequences of GBS toxin receptor in various organisms provides further guidance in preparing preferred fragments. See, for example, Table 4 which compares the amino acid sequence of residues 42 to 536

5

of a human GBS toxin receptor (HP59) (residues 42 to 536 of SEQ ID NO: 8) and a sheep GBS toxin receptor (SP55).

Table 4 Alignment of Human and Sheep GBS Toxin Receptor Amino Acid Sequences

5	Sequences	
SP55	MKSPVSDLAPSDGEEGSDRTPLLQRAPRAEPAPVCCSARYNLAFLSFFGF	50
HP55		50
SP55	FVLYSLRVNLSVALVDMVDSNTTAKDNRTSYECAEHSAPIKVLHNQTGKK	100
HP55	FIVYALRVNLSVALVDMVDSNTTLEDNRTSKACPEHSAPIKVHHNQTGKK	100
SP55	YRWDAETQGWILGSFFYGYIITQIPGGYVASRSGGKLLLGFGIFATAIFT	150
HP55	YQWDAETQGWILGSFFYGYIITQIPGGYVASKIGGKMLLGFGILGTAVLT	150
SP55	LFTPLAADFGVGALVALRALEGLGEGVTYPAMHAMWSSWAPPLERSKLLS	200
HP55	LFTPIAADLGVGPLIVLRALEGLGEGVTFPAMHAMWSSWAPPLERSKLLS	200
SP55	ISYAGAQLGTVVSLPLSGVICYYMNWTYVFYFFGIVGIIWFILWICLVSD	250
HP55	ISYAGAQLGTVISLPLSGIICYYMNWTYVFYFFGTIGIFWFLLWIWLVSD	250
SP55	TPETHKTITPYEKEYILSSLKNQLSSQKSVPWIPMLKSLPLWAIVVAHFS	300
HP55	TPQKHKRISHYEKEYILSSLRNQLSSQKSVPWVPILKSLPLWAIVVAHFS	300
SP55 HP55	YNWTFYTLLTLLPTYMKEVLRFNIQENGFLSAVPYLGCWLCMILSGQAAD	350
	YNWTFYTLLTLLPTYMKEILRFNVQENGFLSSLPYLGSWLCMILSGQAAD	350
SP55	NLRARWNFSTLWVRRVFSLIGMIGPAIFLVAAGFIGCDYSLAVAFLTIST	400
HP55	NLRAKWNFSTLCVRRIFSLIGMIGPAVFLVAAGFIGCDYSLAVAFLTIST	400
SP55	TLGGFCSSGFSINHLDIAPSYAGILLGITNTFATIPGMIGPIIARSLTPE	450
HP55	TLGGFCSSGFSINHLDIAPSYAGILLGITNTFATIPGMVGPVIAKSLTPD	450
SP55	NTIGEWQTVFCIAAAINVFGAIFFTLFAKGEVQNWAISDHQGHRN	495
HP55	NTVGEWQTVFYIAAAINVFGAIFFTLFAKGEVQNWALNDHHGHRH	495
	HP55 - SEQ ID NO: 2	

HP55 - SEQ ID NO: 2 SP55 - SEQ ID NO: 4

Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in a GBS toxin receptor sequence.

Although one class of preferred embodiments are fragments having aminoand/or carboxy-termini corresponding to amino acid positions near functional domains borders, alternative fragments may be prepared. The choice of the aminoand carboxy-termini of such fragments rests with the discretion of the practitioner and will be made based on experimental considerations, such as ease of construction, stability to proteolysis, thermal stability, immunological reactivity, amino- or carboxyl-terminal residue modification, or other considerations. Polypeptide fragments usually contain at least nine amino acids and can contain any number of amino acids provided that the peptide fragment is at least about 80% identical to the corresponding fragment of SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO:8. The human GBS toxin receptor has 41 additional amino acids on the N-terminus compared to the sheep GBS toxin receptor (compare SEQ ID NO:4 and SEQ ID NO:8). Analogs can comprise additions or deletions of some or all of those 41 N-terminal amino acids. N-terminal and C-terminal additions useful, e.g., for purification and/or antibody recognition are also contemplated. Examples include histidine tags, a FLAG (phenylalanine, leucine, alanine, guanine) epitope, fusion partners such as glutathione S transferase, chloramphenicol acetyltransferase (CAT), luciferase, β-galactosidase. and the like. Deletions of unconserved amino acids are also contemplated, provided that the structural integrity and/or binding properties of the GBS toxin receptor are not substantially compromised.

Analogs can also comprise amino acid substitutions, preferably conservative substitutions. Also preferred are conservative and/or non-conservative substitutions in regions having less shared identity among various species. For example, a variant of a GBS toxin receptor can comprise conservative and/or non-conservative — substitutions of amino acids corresponding to residues 2, 6, 10, 11, 16, 17, 24, 31, 44, 46, 52, 53, 55, 74, 75, 81, 82, 84, 93, 102, 132, 133, 137, 144, 145, 148, 149, 155, 159, 163, 165, 166, 179, 212, 219, 235, 236, 239, 242, 246, 253, 254, 257, 259, 260, 271, 283, 285, 319, 324, 332, 333, 338, 355, 362, 366, 377, 439, 442, 445, 450, 453, 461, 487, 488, 491 and 495 of SEQ ID NO:4. Preferably the substitution is an amino acid present in the corresponding position of SEQ ID NO:4 or SEQ ID NO:8. For example, referring to the alignment plot in Table 4, the amino acid corresponding to position 152 of SEQ ID NO:4 can be arginine (R), glutamine (Q), or a conservative or non-conservative substitution of R or Q, and preferably is R or Q. Such regions can

5

10

15

be identified by amino acid sequence alignment plots, such as that shown in Table 4. Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for GBS toxin, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various mutations of a sequence other than the naturally-occurring peptide sequence, such as, for example, single or multiple amino acid substitutions.

A conservative amino acid substitution should generally not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, disrupt 10 disulfide bonds or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles, (1984) Creighton (ed.), W.H. Freeman and Company, New York; Introduction to Protein Structure, (1991), C. Branden and J. Tooze, Garland Publishing, New York, NY; and 15 Thornton et al. (1991) Nature 354: 105 (which are incorporated herein by reference). A conservative substitution is a "replacement of an amino acid in a polypeptide by one with similar characteristics." (McGraw-Hill Dictionary of Scientific and Technical Terms, Fifth Edition, 1994, Sybil P. Parker, Editor in Chief). The structure and characteristics of naturally occurring amino acids has long been known in the art 20 (Biochemistry, Second Edition, Albert L. Lehninger, 1975, pages 71-76) For example, amino acids which are similar by virtue of their hydrophobic R groups are alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, and methionine. Alanine, valine, leucine, and isoleucine are similar by virtue of their aliphatic R groups. Phenylalanine and tryptophan are similar by virtue of their 25 aromatic R groups. Glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine are similar by virtue of their uncharged polar R groups. Glycine and alanine are similar by virtue of their small size. Serine and threonine are similar by virtue of a hydroxyl in their R group. Asparagine and glutamine differ by only one methyl group. Similarly, aspartic acid and glutamic acid differ by only one methyl 30 group, and they are similar by virtue of their acidic R groups. Lysine, arginine, and histidine are similar by virtue of their basic R groups. In addition, lysine and arginine are similar by virtue of the amino groups on the end of the aliphatic chain in their R groups. Tyrosine and phenylalanine are similar by virtue of their aromatic groups.

Amino substitutions commonly made in the art include a substitution of valine for leucine or isoleucine, alanine for glycine, serine for threonine, asparagine for glutamine, aspartic acid for glutamic acid, and lysine for arginine, tyrosine for phenylalanine, and vice versa.

Typically, one skilled in the art would generally refrain from changing amino acids that are conserved among the various GBS toxin receptors, but a conservative substitution might reasonably be made. For example, Table 4 guides one skilled in the art to avoid substitutions, particularly nonconservative substitutions, for amino acids corresponding to residues 1, 3-5, 7-9, 12-15, 18-23, 26-30, 32-43, 45, 47-51, 54, 56-73, 76-80, 83, 85-92, 94-101, 103-131, 134-136, 138-143, 146-147, 150-154, 156-158, 160-162, 164, 167-178, 180-211, 213-218, 220-234, 237-238, 240-241, 243-245, 247-252, 255-256, 258, 261-270, 272-282, 284, 286-318, 320-323, 325-331, 334-337, 339-354, 356-361, 363-365, 367-376, 378-438, 440-441, 443-444, 446-449, 451-452, 454-460, 462-486, 489-490 and 492-494 of SEQ ID NO:4, which are conserved among the GBS toxin receptors shown in Table 4.

Tables 5 and 6 describe sequences within HP59 and SP55, respectively, that match predicted amidation, N-glycosylation, cAMP-phosphorylation, CK2-phosphosylation, myristylation (addition of unsaturated fatty acid molecules), and PKC-phosphosylation sites (Omega 1.1 sequence analysis program). The information contained in these tables provides guidance to one skilled in the art for designing GBS toxin receptor variants and fragments. When designing polypeptide variants, for example, one may decide to avoid substitutions in some or all of these regions. When designing polypeptide fragments other than immunogenic polypeptide fragments, for example, one may opt to include some or all of these regions.

Table 5 Putative Recognition Sites in HP59			Table 6 Putative Recognition Sites in SP55		
Site	Seq. ID NO: 8 Residues:	Sequence	Site	Seq. ID NO: 4 Residues:	Sequence
AMIDATION AMIDATION ASN_GLYCOSYLATION ASN_GLYCOSYLATION ASN_GLYCOSYLATION ASN_GLYCOSYLATION ASN_GLYCOSYLATION ASN_GLYCOSYLATION ASN_GLYCOSYLATION CAMP_PHOSPHO_SITE CK2_PHOSPHO_SITE	23-26 138-141 100-103 112-115 118-121 136-139 266-269 343-346 398-401 297-300 113-116	SGRR TGKK NLSV NTTL NRTS NQTG NWTY NWTF NFST KRIS TTLE	AMIDATION ASN_GLYCOSYLATION ASN_GLYCOSYLATION ASN_GLYCOSYLATION ASN_GLYCOSYLATION ASN_GLYCOSYLATION ASN_GLYCOSYLATION ASN_GLYCOSYLATION ASN_GLYCOSYLATION CK2_PHOSPHO_SITE CK2_PHOSPHO_SITE CK2_PHOSPHO_SITE	97-100 59-62 71-74 77-80 95-98 225-228 302-305 357-360 11-14 73-76	TGKK NLSV NTTA NRTS NQTG NWTY NWTF NFST SDGE TAKD

5

10

15

Table 5 Putative Recognition Sites in HP59			Table 6 Putative Recognition Sites in SP55			
Sia	Seq. ID NO: 8			Seq. ID NO: 4		
Site	Residues:	Sequence	Site	Residues:	Sequence	
CK2_PHOSPHO_SITE	114-117	TLED	CK2_PHOSPHO_SITE	259-262	TPYE	
CK2_PHOSPHO_SITE	300-303	SHYE	CK2_PHOSPHO_SITE	452-455	TIGE	
CK2_PHOSPHO_SITE	493-496	TVGE	MYRISTYL	126-131	GGYVAS	
MYRISTYL	66-71	GAPRAE	MYRISTYL	142-147	GIFATA	
MYRISTYL	167-172	GGYVAS	MYRISTYL	162-167	GALVAL	
MYRISTYL	183-188	GILGTA	MYRISTYL	172-177	GLGEGV	
MYRISTYL	213-218	GLGEGV	MYRISTYL	205-210	GAQLGT	
MYRISTYL	246-251	GAQLGT	MYRISTYL	209-214	GTVVSL	
MYRISTYL	250-255	GTVISL	MYRISTYL	337-342	GCWLCM	
MYRISTYL	378-383	GSWLCM	MYRISTYL	386-391	GCDYSL	
MYRISTYL	427 -4 32	GCDYSL	MYRISTYL	403-408	GGFCSS	
MYRISTYL	444-449	GGFCSS	MYRISTYL	423-428	GILLGI	
MYRISTYL	464-469	GILLGI	MYRISTYL	427-432	GITNTF	
MYRISTYL	468-473	GITNTF	PKC_PHOSPHO SITE	17-19	SDR	
PKC_PHOSPHO_SITE	23-25	SGR	PKC_PHOSPHO_SITE	37-39	SAR	
PKC_PHOSPHO_SITE	58-60	TDR	PKC_PHOSPHO_SITE	55-57	SLR	
PKC_PHOSPHO_SITE	78-80	SAR	PKC_PHOSPHO_SITE	73-75	TAK	
PKC_PHOSPHO_SITE	120-122	TSK	PKC PHOSPHO SITE	97-99	TGK	
PKC_PHOSPHO_SITE	138-140	TGK	PKC_PHOSPHO_SITE	254-256	THK	
PKC_PHOSPHO_SITE	310-312	SLR	PKC PHOSPHO SITE	269-271	SLK	
PKC_PHOSPHO_SITE	317-320	SQK	PKC_PHOSPHO_SITE	276-278	SQK	

In light of the foregoing, preferred polypeptides comprise an amino acid sequence of the formula:

AA1-AAn-AAm

5 wherein:

AA1 is absent or is M;

AAn is a contiguous chain of 0 to 100 amino acids, preferably of 0 or 41 amino acids, even more preferably of residues 2-42 of SEQ ID NO:8; and

AAm is a contiguous chain of 494 amino acids comprising AA43 through AA536, wherein:

10

(1) each of AA43, AA47, AA51, AA52, AA57, AA58, AA65, AA66, AA72, AA85, AA87, AA93, AA94, AA96, AA115, AA116, AA122, AA123, AA125, AA134, AA143, AA173, AA174, AA178, AA185, AA186, AA189, AA190, AA196, AA200, AA204, AA206, AA207, AA220, AA253, AA260, AA276, AA277, AA280, AA283, AA287, AA294, AA295, AA298, AA300, AA301, AA312, AA324. AA326, AA360, AA365, AA373, AA374, AA379, AA396, AA403. AA407, AA418, AA480, AA483, AA486, AA491, AA494, AA502. AA528, AA529, AA532 and AA536 is an essential amino acid or a modified amino acid and preferably is an amino acid residue corresponding to:

> (a) residue 43, 47, 51, 52, 57, 58, 65, 66, 72, 85, 87, 93, 94, 96, 115, 116, 122, 123, 125, 134, 143, 173, 174, 178, 185, 186, 189, 190, 196, 200, 204, 206, 207, 220, 253, 260, 276, 277, 280, 283, 287, 294, 295, 298, 300, 301, 312, 324, 326, 360, 365, 373, 374, 379, 396, 403, 407, 418, 480, 483, 486,

25

15

	491, 494, 502, 528, 529, 532 and 536, respectively, of SEQ ID NO:8;
5	(b) residue 2, 6, 10, 11, 16, 17, 24, 25, 31, 44, 46, 52, 53, 55, 74, 75, 81, 82, 84, 93, 102, 132, 133, 137, 144, 145, 148, 149, 155, 159, 163, 165, 166, 179, 212, 219, 235, 236, 239, 242, 246, 253, 254, 257, 259, 260, 271, 283, 285, 319, 324, 332, 333, 338, 355, 362, 366, 377, 439, 442, 445, 450, 453, 461, 487, 488, 491 and 495, respectively of SEQ ID NO:4;
• •	or
10	(c) a conservative substitution thereof;
	(2) each of AA44-AA46, AA48-AA50, AA53-AA56, AA59-
	AA64, AA67-AA71, AA73-AA84, AA86, AA88-AA92, AA95,
	AA97-AA114, AA117-AA121, AA124, AA126-AA133, AA135-
15	AA142, AA144-AA172, AA175-AA177, AA179-AA184, AA187-
15	AA188, AA191-AA195, AA197-AA199, AA201-AA203, AA205,
	AA208-AA219, AA221-AA252, AA254-AA259, AA261-AA275,
	AA278-AA279, AA281-AA282, AA284-AA286, AA288-AA293,
	AA296-AA297, AA299, AA302-AA311, AA313-AA323, AA325,
20	AA327-AA359, AA361-AA364, AA366-AA372, AA375-AA378,
20	AA380-AA395, AA397-AA402, AA404-AA406, AA408-AA417,
	AA419-AA478, AA481-AA482, AA484-AA485, AA487-AA490, AA492-AA493, AA495-AA501, AA503-AA527, AA530-AA531 and
	AA533-AA535 is
	(a) residue 44-46, 48-50, 53-56, 59-64, 67-71, 73-84,
25	86, 88-92, 95, 97-114, 117-121, 124, 126-133, 135-142, 144-
	172, 175-177, 179-184, 187-188, 191-195, 197-199, 201-203,
	205, 208-219, 221-252, 254-259, 261-275, 278-279, 281-282,
	284-286, 288-293, 296-297, 299, 302-311, 313-323, 325, 327-
	359, 361-364, 366-372, 375-378, 380-395, 397-402, 404-406,
30	408-417, 419-478, 481-482, 484-485, 487-490, 492-493, 495-
	501, 503-527, 530-531 and 533-535, respectively, of SEQ ID
	NO:8; or
	(b) a conservative substitutions thereof; and
	(3) AA315 through AA367 are optionally absent.
35	Preferred polypeptides comprise the amino acid sequence of SEQ ID NO:4,
	SEQ ID NO:8 or an amino acid sequence which varies from that sequence only at the
	specific residues which are not conserved between the sheep GBS toxin receptor

(SEQ ID NO:4) and the human GBS toxin receptor (SEQ ID NO:8). Of those variations, the most preferred variations are those resulting in a polypeptide encoded by SEQ ID NO:11. Even more preferred variations are those amino acids in the 40 corresponding positions of the amino acid sequence of SEQ ID NO:4. Particularly preferred are polypeptides comprising an amino acid sequence that differs from SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:8 at no more than about 20% of the amino acid residues, with increasing preference for no more than about 10%, 5%, 1%, with one to zero amino acid differences being most preferred.

Besides targeting specific amino acids for change, analogs of GBS toxin receptor can also be prepared by techniques involving activity selection, such as, for example, phage display, directed evolution, DNA shuffling, and homologous in procaryotes or eucaryotes of genes from different species, as described in part in U.S. Patent Nos. 5,605,793; 5,830,721; 5,811,238; 5,837,458; 5,093,257; 5,223,409; 5,403,484; 5,571,698; and 5,837,500, which are incorporated herein by reference.

Any variant or fragment of the human and sheep GBS toxin receptors described herein can be tested for the requisite activity by determining whether the variant or fragment can bind GBS toxin.

These polypeptides provide reagents useful in drug discovery and purification and can be used in various in vitro assays, preferably when expressed on the surface of a cell, e.g., a stable transfected cell. For example, assays such as binding assays can be used to screen test compounds, including polysaccharides and other compounds, for their ability to bind the GBS toxin receptor. Assays can identify potential drug candidates that block GBS toxin binding to the GBS toxin receptor. Such drugs are useful for preventing and/or treating early onset disease in neonatal humans. Some polypeptides can be used to competitively inhibit binding GBS toxin to a GBS toxin receptor.

The polypeptides of the invention can be used to affinity purify GBS toxin, a GBS toxin chimeric compound, and other polysaccharides or compounds which can bind the GBS toxin receptor.

The polypeptides can also be used to develop a method of targeting a cytotoxic agent for delivery to a cell that expresses a GBS toxin receptor. For example, a cytotoxic agent can be coupled to a molecule that binds a GBS toxin receptor for selective delivery to the neovasculature of a growing tumor. Such a delivery system would permit a highly concentrated, localized attack on a growing tumor, while minimizing the adverse systemic side effects encountered with most chemotherapeutics. In one instance, the cytotoxic agent can be GBS toxin, which, upon binding to GBS toxin receptor, induces an inflammatory response as described in Hellerqvist et al., Angiogenesis: Molecular Biology, Clinical Aspects, Edited by M.E. Maragoudakis et al., Plenum Press, New York 1994, pp. 265-269. In a similar manner, selective delivery of a therapeutic agent to a cell that expresses a GBS toxin receptor could be used advantageously to treat tumors, rheumatoid arthritis or neural injury, or to facilitate wound healing.

The polypeptides of the invention can also be used to screen for and/or design a GBS toxin mimetic with improved therapeutic properties, such as, for example, improved ability to inhibit hypoxia-induced neovascularization or angiogenesis. Such mimetics are useful in the treatment and prevention of conditions resulting from hypoxia-induced neovascularization or angiogenesis, such as, for example, tumor growth, scarring during wound healing, gliosis during repair of neural injury, reperfusion injury, restenosis, rheumatoid arthritis, psoriasis, other chronic inflammatory diseases characterized by angiogenesis, etc. Therapeutic properties can be improved by enhancing biological stability, affinity for the GBS toxin receptor, complement binding activity, reducing antigenicity, etc.

The polypeptides of the invention can also be used to generate antibodies for various therapeutic and research purposes. The polypeptides of the invention can be used to immunize rabbits, mice, goats, chickens, or other animals known in the art to be amenable to such immunization. Monoclonal antibodies are generally preferred but polyclonal antibodies can also be used, provided that detection of binding of the GBS toxin receptor antibody to the GBS toxin receptor is possible. The production of non-human monoclonal antibodies, e.g., murine, is well known (see, e.g., Harlow et al., Antibodies A Laboratory Manual, Cold Spring Harbor Press, pp. 139-240, 1989,

5

10

15

20

25

incorporated herein by reference). As it may be difficult to generate human monoclonal antibodies to a human receptor or binding domain polypeptide, it may be desirable to transfer antigen binding regions of non-human monoclonal antibodies, e.g. the F(ab')₂ or hypervariable regions or murine monoclonal antibodies, to human constant regions (Fc) or framework regions by recombinant DNA techniques to produce substantially human molecules. Such methods are generally known and are described in, e.g., U.S. Pat. Nos. 4,816,397 and 4,946,778, and EP publications 173,494 and 239,400. Alternatively, one may isolate DNA sequences which code for a human monoclonal antibody or portions thereof that specifically bind to the receptor protein by screening a DNA library from human B cells according to the general protocol outlined in WO 90/14430, and then cloning and amplifying the sequences which encode the antibody (or binding fragment) of the desired specificity.

Usually, polypeptides used for producing antibodies are the full-length receptor or receptor fragments designed from putative extracellular domains identified by a variety of methods known in the art, including computer programs which predict secondary and tertiary structure of a polypeptide based upon its primary amino acid sequence. Another method for designing antigenic peptides utilizes computer programs that predict the high points of hydrophilicity within a particular primary amino acid sequence. For example, using the method of Happ and Woods, *Proc.*Natl. Acad. Sci. USA (1981) 78:3824-3829, via the "Antigen" program in PC/GENE, the inventors identified 3 regions of high hydrophilicity, shown below in Table 7, and used the results to design antigenic peptides to be used in the preparation of antibodies against GBS toxin receptor (see Example 4).

Table 7

25

5

10

High Points of Hydrophilicity in SP55

No.	Ah	Sequence
1	2.05	Glu-Glu-Gly-Ser-Asp-Arg (14-19 of SEQ ID No. 2)
2	1.52	Lys-Asp-Asn-Arg-Thr-Ser (75-80 of SEQ ID No. 2)
3	1.33	Arg-Ala-Pro-Arg-Ala-Glu (25-30 of SEQ ID No. 2)

Ah = Average hydrophilicity.

Antibodies that recognize various portions of the intact GBS toxin receptor can be used to further investigate structure and function of the receptor. The polypeptides of the invention can give rise to antibodies that recognize a variety of forms of GBS toxin receptor, including, but not limited to, intact GBS toxin receptor

expressed on a cell surface, denatured GBS toxin receptor or non-denatured GBS toxin receptor, and GBS toxin receptor purified away from cellular components or GBS toxin receptor contained in a cell lysate. GBS toxin receptor antibodies can be used to study species differences as well as GBS toxin receptor expression levels in various cell types.

Antibodies that recognize a portion or all of an extracellular domain are particularly useful as a diagnostic for the monitoring of tumor growth and metastasis, for the detection or identification of a chronic inflammatory condition, such as, for example, rheumatoid arthritis or psoriasis, and for the detection of other medical conditions arising due to hypoxia-driven angiogenesis, such as, for example, restenosis. Typically, such antibodies can be employed in a variety of standard research and diagnostic techniques, including, but not limited to, western blot, immunoprecipitation, ELISA, radioimmunoassay (RIA), BIACOR®, enzyme-linked-immunoassay (EIA), immunofluorescence, fluorescence activated cell sorting (FACS), and *in vivo* diagnostic imaging systems such as magnetic resonance imaging (MRI), nuclear magnetic resonance (NMR), computerized axial tomography (CAT) scan, and position emission tomography (PET), etc.

In addition, antibodies that block the binding of GBS toxin to a GBS toxin receptor can be used for the treatment or prevention of early onset disease in a neonatal human. Such antibodies can directly or indirectly block the GBS toxin binding site on the GBS toxin receptor.

In one embodiment, the GBS toxin receptor protein is naturally occurring and can be isolated from a cell extract by protein purification techniques known in the art, such as, for example, ion exchange column chromatography, high performance liquid chromatography (HPLC), reversed phase HPLC, or affinity chromatography using antibodies that recognize the GBS toxin receptor.

Alternatively, the isolated proteins and polypeptides are expressed using polynucleotides encoding the polypeptide(s) of the invention in operative association with an appropriate control sequence including a promoter in an expression vector suitable for expression, preferably in a mammalian cell, and also in bacterial, insect, or yeast cells.

Usually, the GBS toxin receptor polynucleotide or a fragment thereof can be expressed in a mammalian system. Such expression will usually depend on a mammalian promoter, which is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. Usually, a promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region typically includes an RNA polymerase binding site and a transcription initiation site.

5

10

15

20

25

Vectors suitable for replication in mammalian cells are known in the art, and can include viral replicons, or sequences that ensure integration of the sequence encoding PAK65 into the host genome. Suitable vectors can include, for example, those derived from simian virus SV40, retroviruses, bovine papilloma virus, vaccinia virus, and adenovirus.

A suitable vector, for example, is one derived from vaccinia viruses. In this case, the heterologous DNA is inserted into the vaccinia genome. Techniques for the insertion of foreign DNA into the vaccinia virus genome are known in the art, and utilize, for example, homologous recombination. The insertion of the heterologous DNA is generally into a gene which is non-essential in nature, for example, the thymidine kinase gene (tk), which also provides a selectable marker. Plasmid shuttle vectors that greatly facilitate the construction of recombinant viruses have been described (see, for example, Mackett et al. (1984); Chakrabarti et al. (1985); Moss (1987)). Expression of the heterologous polypeptide then occurs in cells or individuals which are immunized with the live recombinant vaccinia virus.

Such suitable mammalian expression vectors usually contain one or more eukaryotic transcription units that are capable of facilitating expression in mammalian cells. The transcription unit is comprised of at least a promoter element to mediate transcription of foreign DNA sequences. Suitable promoters for mammalian cells are known in the art and include viral promoters such as those from simian virus 40 (SV40) (Subramani et al., Mol Cell. Biol. 1:854–864, 1981), cytomegalovirus (CMV) (Boshart et al., Cell 41:521–530, 1985), Rous sarcoma virus (RSV), adenovirus (ADV) (Kaufman and Sharp, Mol. Cell. Biol. 2:1304–1319, 1982), and bovine papilloma virus (BPV), as well as cellular promoters, such as a mouse metallothionein-1 promoter (U.S. Patent No. 4,579,821), a mouse VK promoter (Bergman et al., Proc. Natl. Acad. Sci. USA 81:7041–7045, 1993; Grant et al., Nuc. Acids Res. 15:5496, 1987), and a mouse VH promoter (Loh et al., Cell 33:85–93, 1983).

The optional presence of an enhancer element (enhancer), combined with the promoter elements described herein, will typically increase expression levels. An enhancer is any regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to endogenous or heterologous promoters, with synthesis beginning at the normal mRNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or

5

10

15

20

25

flipped orientation, or at a distance of more than 1000 nucleotides from the promoter (Maniatis et al. (1987) Science 236:1237; Alberts et al. (1989) Molecular Biology of the Cell, 2nd ed.). Enhancer elements derived from viruses can be particularly useful, because they typically have a broader host range. Examples useful in mammalian cells include the SV40 early gene enhancer (Dijkema et al (1985) EMBO J. 4:761) and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus (Gorman et al. (1982b) Proc. Natl. Acad. Sci. 79:6777), from human cytomegalovirus (Boshart et al. (1985) Cell 41:521) as well as the mouse μ enhancer (Gillies, Cell 33:717–728, 1983). Additionally, some enhancers are regulatable and become active only in the presence of an inducer, such as a hormone or metal ion (Sassone-Corsi and Borelli (1986) Trends Genet. 2:215; Maniatis et al. (1987) Science 236:1237).

In addition, the transcription unit can also be comprised of a termination sequence and a polyadenylation signal which are operably linked to the GBS toxin receptor coding sequence. Polyadenylation signals include, but are not limited to, the early or late polyadenylation signals from SV40 (Kaufman and Sharp), the polyadenylation signal from the Adenovirus 5 E1B region and the human growth hormone gene terminator (DeNoto et al., Nuc. Acids Res. 9:3719–3730, 1981).

Sequences that cause amplification of the gene may also be desirable, as are sequences which encode selectable markers. Selectable markers for mammalian cells are known in the art, and include, for example, thymidine kinase, dihydrofolate reductase (together with methotrexate as a DHFR amplifier), aminoglycoside phosphotransferase, hygromycin B phosphotransferase, asparagine synthetase, adenosine deaminase, and antibiotic resistant genes such as neomycin.

A GBS toxin receptor, or fragment thereof, can be expressed on the surface of a cell, or can be expressed in soluble or secreted form. Expression on the surface of the cell can be achieved, for example, by including a secretory leader operably linked to a nucleic acid sequence encoding the desired receptor fragment and at least one transmembrane domain. The secretory leader can be that encoded by the GBS toxin receptor gene, or can be a heterologous leader sequence commonly used in the art, such as, for example, the leader sequence of Schizosaccharomyces pombe pho1+ acid phosphatase (Braspenning et al., Biochem Biophys Res. Commun (1998) 245:166-71), the leader sequence of human interleukin-2 (IL-2) gene (Sasada et al., Cell Struct

10

15

20

25

Funct (1988) 13:129-141). Expression in soluble or secreted form can be achieved, for example, by excluding from the gene construct nucleic acid sequences encoding a transmembrane domain. In some instances, solubility and/or secretion are achieved by the use of a fusion partner, such as, for example, chloramphenicol acetyltransferase (CAT), β -galactosidase, and other genes readily expressed in the selected host cell.

The vector that encodes GBS toxin receptor can be used for transformation of a suitable mammalian host cell. Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus and transducing a host cell with the virus or by transfection procedures known in the art, as exemplified by U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (these patents are incorporated herein by reference). The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, 20 HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), N1E-115 (Liles et al., J. Biol. Chem. 261:5307-5313, 1986), PC 12 human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines, such as insect derived cell lines IF9 and IF21. Cell lines of particular preference are those expressing recombinant GBS toxin receptor constructs constitutively, lines which subsequently 25 develop characteristics of a transformed cell, and lines which more preferably express GBS toxin receptor or fragments on the cell surface. Particularly preferred are ECV cells (a bladder carcinoma cell line originally referred to in the scientific literature as an endothelial cell line), human umbilical vein endothelial cells (HUVEC), bovine, 30 sheep, and human adrenal medulla endothelial cells:

Recombinant GBS toxin receptor or fragments thereof can be produced by culturing host cells expressing the receptor or fragment in a suitable culture medium and under appropriate cell culture conditions. Culture media and conditions are

5

10

variable depending on the requirements of a particular host cell line and are well-known in the art. Typically, cells are cultured at 37°C in a cell culture incubator with a fixed amount of C02, usually in the range of 5-10%.

In another embodiment, the polypeptide fragments can be synthesized chemically by techniques well known in the art, such as solid-phase peptide synthesis 5 (Stewart et al., Solid Phase Peptide Synthesis, W.H. Freeman Co., San Francisco (1963)); Merrifield, J Am Chem Soc 85:2149-2154 (1963)). These and other methods of peptide synthesis are also exemplified by U.S. Patent Nos. 3,862,925, 3,842,067, 3,972,859, and 4,105,602. The synthesis can use manual synthesis techniques or automatically employ, for example, an Applied BioSystems 430A or 431A Peptide 10 Synthesizer (Foster City, California) following the instructions provided in the instruction manual supplied by the manufacturer. It will be readily appreciated by those having ordinary skill in the art of peptide synthesis that the intermediates which are constructed during the course of synthesizing the present analog compounds are themselves novel and useful compounds and are thus within the scope of the 15 invention.

In addition to polypeptides consisting only of naturally-occurring amino acids, peptidomimetics are also provided. Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compounds are termed "peptide 20 mimetics" or "peptidomimetics" (Fauchere, J. (1986) Adv. Drug Res. 15: 29; Veber and Freidinger (1985) TINS p.392; and Evans et al. (1987) J. Med. Chem 30: 1229, which are incorporated herein by reference) and are usually developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or 25 prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biochemical property or pharmacological activity) but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: -CH2NH-, -CH2S-, -CH2-CH2-, -CH=CH-(cis and trans), -COCH2-, -CH(OH)CH2-, and -CH2SO-, by methods known in the art 30 and further described in the following references: Spatola, A.F. in "Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins," B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983); Vol. 1, Issue 3, "Peptide Backbone Modifications" (general review); Morley, J.S., Trends Pharm Sci (1980) pp. 463-468 (general

review); Hudson, D. et al., Int J Pept Prot Res (1979) 14:177-185 (-CH2NH-, CH2CH2-); Spatola, A.F. et al., Life Sci (1986) 38:1243-1249 (-CH2-S); Hann, M.M., J Chem Soc Perkin Trans I (1982) 307-314 (-CH-CH-, cis and trans); Almquist, R.G. et al., J. Med Chem (1980) 23:1392-1398 (-COCH2-); Jennings-White, C. et al., Tetrahedron Lett (1982) 23:2533 (-COCH2-); Szelke, M. et al., 5 European Appln. EP 45665 (1982) CA: 97:39405 (1982) (-CH(OH)CH2-); Holladay, M.W. et al., Tetrahedron Lett (1983) 24:4401-4404 (-C(OH)CH2-); and Hruby, V.J., Life Sci (1982) 31:189-199 (-CH2-S-); each of which is incorporated herein by reference. A particularly preferred non-peptide linkage is -CH2NH-. Such peptide mimetics may have significant advantages over polypeptide embodiments, including, 10 for example: more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (e.g., a broad-spectrum of biological activities), reduced antigenicity, and others. Labeling of peptidomimetics usually involves covalent attachment of one or more labels, directly or through a spacer (e.g., an amide group), to non-interfering 15 position(s) on the peptidomimetic that are predicted by quantitative structure-activity data and/or molecular modeling. Such non-interfering positions generally are positions that do not form direct contacts with GBS toxin (e.g., are not contact points in the GBS toxin binding domain of the GBS toxin receptor). Derivitization (e.g., 20 labelling) of peptidomimetics should not substantially interfere with the desired biological or pharmacological activity of the peptidomimetic.

Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch (1992) Ann. Rev. Biochem. 61: 387, incorporated herein by reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

The invention also provides a complex comprising a GBS toxin bound to a mammalian GBS toxin receptor or a fragment of a mammalian GBS toxin receptor. Preferably, the complex comprises a GBS toxin bound to a GBS toxin receptor polypeptide described above that can bind GBS toxin. Typically, a complex is formed by contacting a GBS toxin with such a polypeptide under conditions that

25

permit specific binding of the GBS toxin to the polypeptide. The GBS toxin can be labeled or unlabeled. The polypeptide can be present on the surface of a cell, or immobilized in a well or on a bead, or the polypeptide can be present in solution.

5 DETECTION METHODS

10

15

20

25

30

Yet another aspect of the invention provides methods for detecting or monitoring a variety of medical conditions characterized by pathologic and/or hypoxia-driven angiogenesis or neovascularization. Examples include, but are not limited to, early onset disease in the neonate, and the progression of cancers involving tumors.

Early onset disease can be diagnosed by detecting the presence or absence of GBS toxin in a patient. One method of detection is a competition assay that determines the effect of a suspected sample on the formation of a complex between GBS toxin and a GBS toxin receptor or fragment thereof. For example, the method comprises contacting a control GBS toxin with a GBS toxin receptor polypeptide, in the presence and absence of a sample suspected of containing GBS toxin and under conditions that permit specific binding of the GBS toxin to the polypeptide, and comparing the amount of complex formation achieved in the presence of the suspected sample to the amount of complex formation achieved in the absence of the suspected sample. Preferably, the control GBS toxin is substantially purified and of a known concentration. Preferably, the control GBS toxin further comprises a label. Suitable labels include, but are not limited to, radioisotopes, chromophores, fluorophores, biotin, avidin, and other labels used by one skilled in the art. Another method directly measures, rather than by competition with a control GBS toxin, complex formation between GBS toxin present in a suspected sample and a GBS toxin receptor polypeptide.

Pathologic vasculature can be detected in a mammalian tissue by detecting the presence or absence of GBS toxin receptor in the region of a tumor, with the presence of GBS toxin receptor being indicative of the presence of pathologic vasculature. The method can be used to monitor tumor growth or metastasis. One method of detection involves the use of molecules, e.g. antibodies, that specifically bind to a GBS toxin receptor, preferably an extracellular domain of GBS toxin receptor. Typically, the method comprises administering, to a mammalian tissue, e.g. in a mammal having a cancerous tumor, e.g., an antibody that recognizes a GBS toxin receptor, and

detecting specific binding of the antibody. Typically, the antibody is a labeled antibody. Preferably, the observations are quantitative and can be visual.

During surgery, the margin of a tumor can be visualized by any of a number of imaging techniques known in the art and described above. The imaging of the tumor is effected by detecting the binding of a labeled antibody or other molecules to the GBS toxin receptor on the pathologic vasculature of a tumor. This type of surgery is also known as virtual surgery because while performing the surgery, the surgeon views the tumor indirectly on an imaging screen.

10 DRUG DISCOVERY

5

15

20

25

30

A fourth aspect of the invention provides methods, using the polypeptides of the invention, of identifying drug candidates for the treatment of medical conditions characterized by hypoxia-driven angiogenesis or neovascularization. Preferred compounds are competitive inhibitors of GBS toxin binding to a GBS toxin receptor or inhibit GBS toxin receptor activity. Particularly preferred are compounds that inhibit the first phosphorylation step in the signal transduction pathway. Compounds can be produced by a variety of random drug design methods commonly known in the art, such as, for example, combinatorial chemistry (U.S. Patent No. 5,646,285; U.S. Patent No. 5,639,603), peptide libraries (U.S. Patent No. 5,591,646; U.S. Patent No. 5,367,053; U.S. Patent No. 5,747,334), phage display (U.S. Patent No. 5,403,484; U.S. Patent No. 5,223,409), SELEX® (U.S. Patent No. 5,773,598; U.S. Patent No. 5,763,595; U.S. Patent No. 5,763,566), and combinatorial carbohydrate chemistry (Hirschmann et al., J Med Chem (1996) 39:2441-2448; Hirschmann et al., J Med Chem (1998) 41:1382-1391; Sofia MJ, Mol Divers (1998) 3:75-94; U.S. Patent No. 5,780,603; U.S. Patent No. 5,756,712)

An alternative approach is rational drug design with the intent of producing a GBS toxin mimetic or a GBS toxin receptor mimetic with improved therapeutic properties using techniques such as x-ray crystallography, nuclear magnetic resonance (NMR) correlation spectra (U.S. Patent No. 5,698,401), computer assisted molecular modeling (U.S. Patent No. 5,579, 250; U.S. Patent No. 5,612,895; U.S. Patent No. 5,680,331, Cooper et al., J. Comput.-Aided Mol. Design, 3:253-259 (1989); Brent et al., J. Comput.-Aided Mol. Design 2:311-310 (1988)) and other methods of rational drug design known in the art. FIG. 1 provides a broad overview of some of the main steps in some of the rational drug design methods of the present invention. For

example, one approach to rational drug design involves a computer program, such as INSIGHTII (available from Bisoym Technologies, 10065 Barnes Canyon Road, San Diego, California) to identify active sites in proteins by homology-based modeling. This method facilitates the modeling of a protein by using a similar protein whose structure is well known. Commercial software containing search algorithms for three dimensional database comparisons are available from vendors such as Day Light Information Systems, Inc., Irvine, California 92714, and Molecular Design Limited, 2132 Faralton Drive, San Leandro, California 94577.

In one embodiment, the compound can bind the GBS toxin receptor and induce an inflammatory response in a manner similar to the binding of GBS toxin to the GBS toxin receptor. Such compounds can be used, for example, as a drug to target an inflammatory response to the developing vasculature of a tumor.

In another embodiment, the compound can bind the GBS toxin receptor with or without inducing an inflammatory response, preferably without inducing an inflammatory response. In one instance, the compound can be used as a vehicle to target pathological neovasculature for treatment with a cytotoxic agent. For example, the cytotoxic agent can be chemically coupled to the compound to form a chimeric drug. Such chimeric drugs can be used in the treatment of tumors, rheumatoid arthritis, wound healing, spinal cord injury, and other conditions characterized by hypoxia-driven angiogenesis or neovascularization. In another instance, the compound can be used directly to competitively inhibit binding of GBS toxin to a GBS toxin receptor. Such compounds can be used in the treatment of early-onset disease in the neonate.

In a third embodiment, the compound can bind GBS toxin and can be used in the treatment of early-onset disease in the neonate.

The polynucleotides of the invention can be expressed in random mutagenesis systems such as phage display or the yeast two-hybrid system for the synthesis and identification of mutant peptide GBS toxin receptor polypeptides that bind GBS toxin. Alternatively, immobilized or soluble GBS toxin receptor fragments of the invention can be used to screen combinatorial peptide and combinatorial chemical libraries and non-random recombinant and synthetic peptides and other compounds (such as non-peptide molecules) for GBS toxin receptor binding. Compounds that bind GBS toxin or GBS toxin receptor can then be further characterized in a functional assay for any of the activities described above in order to identify a drug candidate for the treatment

30

5

10

15

of medical conditions involving angiogenesis or neovascularization.

A compound which inhibits binding of GBS toxin to a GBS toxin receptor can be identified by combining a test compound with a mammalian GBS toxin receptor or fragment thereof capable of binding GBS toxin, under conditions that permit specific binding of GBS toxin to the GBS toxin receptor or fragment, and determining the amount of inhibition by the compound of the binding of GBS toxin to the GBS toxin receptor or fragment.

In a preferred embodiment, the GBS toxin receptor or fragment is expressed by a cell, preferably on the cell surface. The cells are contacted with labeled GBS toxin in the presence or absence of the test compound. A change in the binding of GBS toxin to the GBS toxin receptor is then determined. Alternatively, the GBS toxin is unlabeled and an antibody that recognizes GBS toxin is labeled instead. The labeled antibody is used to measure inhibition by a compound of GBS toxin binding to the GBS toxin receptor or fragment. In another embodiment, the GBS toxin receptor or fragment is not associated with a cell, but is instead coupled to a matrix, such as, for example, a well in a microtiter plate or a bead. Additional suitable solid supports include latex, polystyrene beads (Interfacial Dynamics Corp. Portland, Oreg.), magnetic particles (Advanced Magnetics, Cambridge, Mass.) and nylon balls (Hendry et al., J. Immunological Meth., 35:285-296, 1980). The receptor or fragment can be coupled to the matrix directly or indirectly through an antibody, coupled to the matrix, that binds the receptor fragment. In a third embodiment, the GBS toxin receptor or fragment is soluble and can be immunoprecipitated with an antibody that recognizes the receptor or fragment.

A preferred method for identifying a compound which binds a mammalian GBS toxin receptor comprises the steps of (1) combining a test compound with a GBS toxin receptor or fragment thereof under conditions that allow specific binding to occur, and (2) detecting a complex formed between the test compound and the GBS toxin receptor or fragment. A preferred method is a competition assay which determines the ability of the test compound to compete for binding to the GBS toxin receptor or fragment. In such an assay, GBS toxin is combined with the GBS toxin receptor or fragment in the presence or absence of the test compound. Decreased specific binding of GBS toxin in the presence versus the absence of the test compound is indicative of the ability of the test compound to bind a mammalian GBS toxin receptor. Another method comprises combining a control compound with the GBS

5

10

15

20

25

toxin receptor or fragment under the same conditions as the test compound and comparing the amount of complex formation between the test compound or the control compound and the GBS toxin receptor or fragment thereof. Preferably, the test compound and/or the control compound are labeled. The test compound can be any of a number of classes of compounds, such as for example, small organic molecules (such as those used for and obtained by combinatorial chemistry), polysaccharides, polypeptides, RNA, antibodies, and single chain antibodies. In a preferred embodiment, the polypeptide is expressed by a cell, preferably on the surface of the cell, and preferably by a stable transfected cell. Such a system is particularly useful for testing the effectiveness of a chimeric compound comprising a cytotoxic agent. The cytotoxic activity of the compound can be determined by exposing a cell expressing the GBS toxin receptor on the cell surface to the test chimeric compound and detecting signs of cytotoxicity. One could detect such signs by a viability stain of the cell, by detecting apoptosis (for example, by a DNA ladder assay or a TUNELTM stain, which binds to broken DNA), by measuring tritiated thymidine incorporation into the cell, and by quantitating kinase-dependent phosphorylation (e.g., using phosphoantibodies or various phosphoimaging techniques).

In another embodiment, the invention provides a method for identifying an inhibitor of GBS toxin receptor. The method comprises incubating test cells in the presence and absence of a test compound. The test cells express GBS toxin receptor or a fragment thereof having GBS toxin receptor activity (e.g., a fragment that increases the proliferation or migration of the expressing cells relative to control cells of the same cell type that do not express the fragment). The test cells are incubated under conditions in which the cells incubated in the absence of the test compound can proliferate or migrate. Control cells that do not express the GBS toxin receptor or fragment proliferate or migrate less than cells that express the GBS toxin receptor or fragment. The proliferation or migration (also referred to herein as motility) of the test cells incubated in the presence or absence of the test compound is compared. Less proliferation or migration in the presence of the test compound than in the absence of the test compound is indicative of the test compound being an inhibitor of the GBS toxin receptor. Preferably, as a control to determine whether the test compound specifically inhibits the GBS toxin receptor, the proliferation or migration of control cells in the presence and absence of the test compound is also compared. In the

5

10

15

20

25

absence of a difference in the proliferation or migration of control cells incubated in the presence or absence of the test compound, decreased proliferation or migration in test cells exposed to the test compound relative to test cells not exposed to the test compound is indicative of specific inhibition of the GBS toxin receptor. It will be readily apparent that the control portions of the method need not be performed contemporaneously with the test portions of the method. For example, control cells can be incubated with a battery of test compounds to determine cellular effects of the test compounds prior to incubating the test cells with the test compounds. Motility or migration can be determined by detecting movement of cells on a culture dish. Proliferation can be detected in a number of ways, including, but not limited to, measuring tritiated thymidine incorporation, cell counts, apoptosis assays, and viability assays. Preferred cells include cells transfected with GBS toxin receptor. preferably endothelial cells transfected with GBS toxin receptor, even more preferably vascular endothelial cells or microvascular endothelial cells. Primary cells that express GBS toxin receptor are also preferred, for example, endothelial cells that have been passaged in cell culture, at confluence, no more than 8 or 9 times. A preferred class of test compounds includes kinase inhibitors, preferably cAMPdependent kinase inhibitors, PKC inhibitors, and CK2 inhibitors, which can be used as a starting point for developing more specific GBS toxin receptor inhibitors. Another class of compounds includes antibodies specific for GBS toxin receptor. Particularly preferred are single chain antibodies, preferably a collection of single chain antibodies that recognize various epitopes on the GBS toxin receptor. Less preferred are divalent antibodies specific for the binding site of the GBS toxin

receptor ligand because they may trigger the signal transduction cascade upon dimerization. 25

Another embodiment of the invention is a method of identifying an inhibitor of endothelial cell proliferation or migration, which are essential components of angiogenesis. The method basically comprises the steps described in the preceding paragraph and uses endothelial cells.

Yet another embodiment of the invention is a method of identifying a therapeutic compound for the treatment or prevention of a medical condition characterized by pathologic or hypoxia-driven angiogenesis or neovascularization. The method basically comprises the steps described above and uses cells from tissues derived from mammals afflicted with the medical condition or cells that serve as a

5

10

15

20

model for afflicted tissue.

5

10

15

20

A preferred method for designing a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor comprises (1) simulating and selecting the most probable conformations of a GBS toxin receptor or fragment thereof, (2) designing a chemically modified analog that substantially mimics the energetically most probable three-dimensional structure of the GBS toxin receptor or fragment, (3) chemically synthesizing the analog, and (4) evaluating the bioactivity of the analog. Preferably, steps (a) and (b) are performed with the aid of a computer program.

A preferred method for designing a compound which binds to a mammalian GBS toxin receptor comprises (1) simulating and selecting the most probable conformations of a GBS toxin receptor or fragment thereof, (2) deducing most probable binding domains of the receptor or fragment, (3) designing a compound that would form the energetically most probable complexes with the receptor or fragment, (4) chemically synthesizing the compound, and (5) evaluating the bioactivity of the compound. Preferably, steps (a)-(c) are performed with the aid of a computer program.

Preferred polypeptides for use in the screening assays described above are polypeptides sharing at least about 85% identity, preferably at least about 95% identity, and most preferably greater than about 99% identity with the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4, or a fragment thereof having GBS toxin receptor activity. Most preferred are polypeptides having an amino acid sequence of SED ID NO: 2, 4 OR 8 or a fragment thereof having GBS toxin receptor activity.

25 Methods Of Purification

Another aspect of the invention is a method for purifying a compound that binds a GBS toxin receptor, for example, natural ligand, other polysaccharides, or an antibody specific for the GBS toxin receptor. The method comprises providing a polypeptide comprising a mammalian GBS toxin receptor or fragment thereof that binds GBS toxin, contacting the polypeptide with a sample comprising the compound under conditions that allow specific binding of the compound to the polypeptide, and separating the bound compound from the remainder of the sample. The polypeptide can be soluble but preferably is immobilized on a substrate e.g., on a bead, membrane or on the surface of a cell, preferably a stable transfected cell.

METHODS OF TREATMENT

5

GBS toxin receptor polypeptides and antibodies that interfere with GBS toxin binding can be used in a method of treatment of the human or animal body. For example, such inhibitors of GBS toxin binding can be administered to a patient to treat or prevent medical conditions involving GBS toxin binding to a GBS toxin receptor, such as, for example, early onset disease in the neonate.

GBS toxin mimetics or other compounds that bind and/or inhibit GBS toxin receptor, some of which can be identified by the drug discovery assays of the invention, can be used in a method of treatment of the human or animal body or can be used for the manufacture of a medicament for the treatment or prevention of any of a number of medical conditions involving pathologic and/or hypoxia-driven angiogenesis, such as, for example, cancerous tumors, chronic inflammatory diseases, scarring during wound healing or repair of neural injury.

In a preferred embodiment, such a compound exerts its therapeutic effect by binding GBS toxin receptor and evoking an inflammatory response, as does GBS toxin. Preferably, such compounds comprise a sulfhydryl, hydroxyl, or amino group displayed so as to be available for binding complement C3.

In another preferred embodiment, the compound is an inhibitor of GBS toxin activity. Preferred inhibitors include, but are not limited to, kinase inhibitors, single chain antibodies specific for the GBS toxin receptor, and antisense polynucleotides that specifically hybridize under high stringency conditions to a GBS toxin receptor nucleic acid sequence, such as that of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:7.

In another preferred embodiment, the compound exerts its therapeutic effect without evoking an inflammatory response. The compound can be used to deliver a cytotoxic agent to tissue in close proximity to a cell expressing a GBS toxin receptor, such as, for example, a tumor undergoing angiogenesis. Preferably, the compound is covalently attached to a cytotoxic agent and can be associated non-covalently with a cytotoxic agent, such as, for example, on the external surface of a liposome, micelle, or other lipophilic drug encapsulating structure. Preferred cytotoxic agents include antineoplastic agents commonly known in the art, such as, for example,

mechlorethamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide, and other alkylating agents, methotrexate and other folate antagonists, 6-mercaptopurine and other purine antagonists, 5-fluorouracil and other pyrimidine antagonists, cytarabine, ovinblastine, vincustine, and other vincas, etoposide and other podophyllotoxins, doxorubicin, bleomycin, mitomycin, and other antibiotics, carmustine, lomustine and other nitrosureas, cisplatin, interferon, asparaginase, tamoxifen, flutamide, and taxol. Other preferred biologic agents include sense and/or antisense RNA or DNA sequences derived from specific tumor promoter or suppressor genes, such as, for example, the p53 and TGF gene families, signal transduction protein family members such as, for example, ras and myc, and growth factor receptor kinases such as, for

5

10

15

20

25

example flt2 and flk1, Tai1, Tai2, and neuropholin, and other genes implicated in neoplastic disease and other diseases driven by pathologic angiogenesis.

In another embodiment, GBS toxin receptor polypeptide or fragment thereof can be administered to a subject as a decoy to reduce the amount of stimulation of the GBS toxin receptor present in afflicted tissues (e.g., tumor tissues), thereby reducing cellular responses leading to proliferation and migration of cells of the afflicted tissues. Preferably, the GBS toxin receptor polypeptide or fragment is administered in soluble form, even more preferably sans transmembrane domains.

10 PHARMACEUTICAL COMPOSITIONS

5

15

20

25

30

Polypeptides of the invention that comprise a domain essential for GBS toxin binding that have the desired characteristics for bioavailability, stability and other important parameters of pharmacokinetics *in vivo* can be used as a competitive inhibitor of GBS toxin binding for medical conditions, such as, for example, early onset disease in the neonate, in which GBS toxin binding is undesirable. Appropriate polypeptides can include fragments having an amino acid sequence corresponding to a partial or full sequence of SEQ ID NO: 2 or SEQ ID NO: 4 or analogs thereof.

Compounds determined by assays using the polypeptides of the invention to bind and/or GBS toxin receptor and/or induce an inflammatory response, and that have the desired pharmacokinetic characteristics, can be used as treatments for medical conditions in which GBS toxin binding can be therapeutic, such as, for example, medical conditions involving pathologic or hypoxia-driven angiogenesis or neovascularization.

Pharmaceutical compositions of the invention include a pharmaceutically acceptable carrier that may contain a variety of components that provide a variety of functions, including regulation of drug concentration, regulation of solubility, chemical stabilization, regulation of viscosity, absorption enhancement, regulation of pH, and the like. For example, in water soluble formulations the pharmaceutical composition preferably includes a buffer such as a phosphate buffer, or other organic acid salt, preferably at a pH of between about 7 and 8. Other components may include antioxidants, such as ascorbic acid, hydrophilic polymers, such as, monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, dextrins, chelating agents, such as EDTA, and like components well

known to those in the pharmaceutical sciences, e.g. Remington's Pharmaceutical Science, latest edition (Mack Publishing Company, Easton, PA).

An effective amount of an active compound such as a GBS toxin receptor polypeptide, mimetic or analog, or GBS toxin mimetic or analog for particular applications depends on several factors, including the chemical nature of the polypeptide, mimetic or analog, the disorder being treated, the method of administration, and the like. Preferably, an effective amount will provide a concentration of polypeptide or mimetic of between about 0.0001 to $100 \mu M$ at the target GBS toxin receptor on a cell surface, more preferably less than $10 \mu M$, with less than $1 \mu M$ being most preferred.

The active compound can be administered to a mammalian host in a variety of forms, i.e., they may be combined with various pharmaceutically acceptable inert carriers in the form of tablets, capsules, lozenges, troches, hard candies, powders, sprays, elixirs, syrups, injectable or eye drop solutions, and the like depending on the chosen route of administration, e.g., orally or parenterally. Parenteral administration in this respect includes administration by the following routes: intravenous, intramuscular, subcutaneous, intraocular, intrasynovial, transepithelial (including transdermal, ophthalmic, sublingual and buccal), topical (including ophthalmic, dermal, ocular, rectal, nasal inhalation via insufflation and aerosol), and rectal systemic.

The active compound may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, it may be enclosed in hard or soft shell gelatin capsules, compressed into tablets, or incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at lease 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2% to about 6% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 1 and 1000 mg of active compound.

5

10

15

20

25

Tablets, troches, pills, capsules and the like may also contain the following: a binder such as polyvinylpyrrolidone, gum tragacanth, acacia, sucrose, corn starch or gelatin; an excipient such as calcium phosphate, sodium citrate and calcium carbonate; a disintegrating agent such as corn starch, potato starch, tapioca starch, certain complex silicates, alginic acid and the like; a lubricant such as sodium lauryl sulfate, talc and magnesium stearate; a sweetening agent such as sucrose, lactose or saccharin; or a flavoring agent such as peppermint, oil of wintergreen or cherry flavoring. Solid compositions of a similar type are also employed as fillers in soft and hard-filled gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye, flavoring such as cherry or orange flavor, emulsifying agents and/or suspending agents, as well as such diluents as water, ethanol, propylene glycol, glycerin and various combinations thereof. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

The active compound may also be administered parenterally or intraperitoneally. For purposes of parenteral administration, solutions in sesame or peanut oil or in aqueous propylene glycol can be employed, as well as sterile aqueous solutions of the corresponding water-soluble, alkali metal or alkaline-earth metal salts previously enumerated. Such aqueous solutions should be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. Solutions of the active compound as a free base or a pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. A dispersion can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal injection purposes. In

5

10

15

20

25

this connection, the sterile aqueous media employed are all readily obtainable by standard techniques well-known to those skilled in the art.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of a dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by use of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying technique which yield a powder of the active ingredient plus any additional desired ingredient from the previously sterile-filtered solution thereof.

For purposes of topical administration, dilute sterile, aqueous solutions (usually in about 0.1% to 5% concentration), otherwise similar to the above parenteral solutions, are prepared in containers suitable for drop-wise administration to the eye. The compounds of this invention may be administered to a mammal alone or in combination with pharmaceutically acceptable carriers. As noted above, the relative proportions of active ingredient and carrier are determined by the solubility and

5

10

15

20

25

chemical nature of the compound, chosen route of administration, the particular compound chosen and the physiological characteristics of the particular patient under treatment.

5 Kits

10

15

Yet another aspect of the invention is a kit for use in carrying out any of the above methods. A preferred embodiment is a kit comprising a GBS toxin receptor or fragment thereof. Preferably, the receptor or fragment is immobilized. A preferred kit can be used for identifying a compound that binds to GBS toxin receptor, and comprises at least one cell that expresses GBS toxin receptor.

Another embodiment is a kit for monitoring tumor growth or metastasis, comprising a reagent for detecting expression of a GBS toxin receptor. Examples of such reagents include, but are not limited to, polynucleotide probes that hybridize to a GBS toxin receptor nucleic acid sequence and compounds that bind to a GBS toxin receptor, such as, for example, an antibody that specifically recognizes GBS toxin receptor, a GBS toxin, a GBS toxin mimetic, or other compounds identified by the screening methods described above.

A third embodiment is a kit for purifying a compound that binds a GBS toxin receptor, comprising a GBS toxin receptor or fragment thereof that binds the compound. Preferred compounds include GBS toxin, GBS toxin mimetics, antibodies that specifically bind GBS toxin receptor, and other compounds identified by the screening methods described above.

Additional kit components can include, but are not limited to, additional reagents required for detection, a reference standard(s), instructions for use, and the like. Suitable reference standards include positive controls, negative controls, photographs of such controls, tabulated or graphed data of such controls, and the like. The kits may further comprise instructions for carrying out the methods described above, preferably printed instructions.

EXAMPLES

EXAMPLE 1 - CLONING SHEEP GBS TOXIN RECEPTOR

Primary culture of sheep lung endothelial cells

Small pieces of primary lung tissues from a 7-week old sheep are cut into small pieces in Hank's balanced salt solution (HBSS) containing 10 mM HEPES buffer (Life Technology), 1% penicillin/streptomycin and 0.1% gentamycin, and are cultured in sheep lung complete medium (Life Technology) at 37°C. After one week of the culture, clones of sheep lung endothelial cells are identified by Cobblestone morphology and harvested into 24-well tissue culture plates (Falcon) using cloning rings. When the cells are confluent, they are detached by pancreatin and transferred to a 60-mm tissue culture Petri dish or a T-25 tissue culture flask (Falcon). When they are confluent again, they are split and cultured into a few 100-mm tissue culture plates (Falcon). Each split is considered to be one passage. The same procedure is repeated until enough cells (~10⁸) are obtained for isolation of mRNA.

Isolation of mRNA and construction of cDNA library

Poly(A)+ RNA is isolated from 9.2 x 10⁷ sheep lung endothelial cells (passage 8 and 9) by a standard method (Pharmacia). A total of 16 μg poly(A)⁺ RNA is acceptable amount obtained. 2.5 μg mRNA can be used to construct a cDNA library. Poly(A)⁺ RNA is oligo(dT)-primed (with *Not* I restriction site) and converted into double-stranded cDNA. After adding a *BstX I/EcoR* I adaptor, the cDNA is unidirectionally cloned into the *BstX* I and *Not* I sites of pCDNA3.1(+) (Invitrogen).

5

10

15

20

25

E. coli Top10F' (Invitrogen) is used as a host strain for amplification. 5.38×10^6 primary clones are an acceptable number generated. The library is amplified by plating cells onto fifty large LB agar plates containing ampicillin ($100 \mu g/ml$). The plates are scraped and aliquoted so that each aliquot represents 10 plates. DNA is purified by Qiagen Max columns (Qiagen).

Screening of cDNA library for a gene encoding GBS toxin receptor

To screen a cDNA library for a gene encoding GBS toxin receptor
gene, a unique colorimetric method is used. Five µg plasmid DNA from each pool of
cDNA library is used to transfect COS7 cells. The transfected cells are cultured in
four to eight 96-well tissue culture plates (Falcon) for transient expression. Each well
contains about 20,000 transfected cells in DMEM medium (Life Technology). COS7
cells transfected with pCDNA3.1(+) are used as a control. After 3 days expression,
the medium is carefully removed. Each well is rinsed 3 times with HPSS buffer
containing Mg²⁺ and Ca²⁺ (wash buffer) (Life Technology).

The cells are then incubated with biotinylated toxin (50μ l per well; 1 to 1.5 μ g/ml) at room temperature for 1 h. After the hour incubation, the biotinylated toxin is discarded and the wells are rinsed 3 times with the wash buffer. The cells are incubated with streptavidin- β -gal solution and each well is rinsed 3 times with the wash buffer. The cells are then incubated with PNPG (50μ l per well; 1 mg/ml in substrate buffer) at 37°C. Absorbance at 405 nm is measured by an ELISA reader at 1 and 20 h, respectively. The cells which give the highest OD are harvested. Plasmid DNA is isolated by Hirt extraction. Plasmid DNA is amplified in *E. coli* to have enough DNA for the next transfection (enrichment).

Enrichment is done 8 times by this colorimetric method. The number of the transfected cells loaded into each well is gradually decreased in the last few enrichments and untransfected cells are added to each well to give a total number of 20,000 cells per well for the cells to be confluent and to reduce background after 3 days' expression. At the last enrichment, each well has only 1 to 10 transfected cells. Cells giving the highest OD are harvested. DNA is isolated and amplified in *E. coli*.

A number of isolated clones are individually assayed by this colorimetric method. The clones which showed higher binding to CM101 are sequenced.

Sequence analysis

5

10

15

20

25

DNA sequence analysis of clone pFU102, which has a 2.1kb insert, revealed a sequence encoding a partial integral glycoprotein. N-terminal sequence was obtained by 5'RACE method (Life Technology) and a full-length gene is designated as SP55. Triple ligation yielded pCD55, which contains an entire coding region of SP55.

mRNA for the SP55 has 2844 nucleotides, encoding a protein of 495 amino acids with a predicated mass of 55 KDa, SP55. Analysis by the method of Klein et al. (Klein et al., *Biochim Biophys Acta*, 815:468-476 (1985)) classifies SP55 as an integral protein with seven transmembrane segments. SP55 has both N-glycosylation and kinase phosphorylation sites. A Swiss-Prot. search of SP55 did not reveal any high homology to known human proteins. However, SP55 has some identity (~ 30%) to renal sodium-dependent phosphate transporters from human, rabbit, mouse and rat. In addition, SP55 has some identity (~ 30 to 39%) to hypothetical proteins (HYP50 and HYP63) from *C. elegans*.

15

20

25

30

5

10

Example 2 - Cloning Human GBS Toxin Receptor

The sheep GBS toxin receptor sequence shares about 37% identity with HYP50 and about 33% identity to HYP63, two hypothetical proteins from C. elegans. In the regions corresponding to amino acid residues 180-186 and 443-449 of SEQ ID No. 2, five amino acids within a seven amino acid stretch are absolutely conserved among the three proteins.

A first degenerate oligonucleotide, CMR3-S: 5'-CGGGATCCCGCCNGCNATGCAYRSHRTSTGG-3'(SEQ ID No. 5), was designed to include all-possible codons encoding the amino acid sequences of SP55, HYP50, and HYP63 in the 180-186 region. A second degenerate oligonucleotide, CMR4-AS2: 5'-GGAATTCCDGGDGCRATKTCNARRTRRTT-3' (SEQ ID No. 6), was designed to include the complementary sequences of all possible codons encoding the amino acid sequences of SP55, HYP50, and HYP63 in the 443-449 region.

Polymerase chain reaction (PCR) was conducted using these oligonucleotides and a human embryo lung cDNA library as a template. The reaction yielded three overlapping sequences approximately 400 bp in size, which encompass part of the nucleic acid sequence of SEQ ID No. 3. These sequences were then used as probes to clone the remainder of the gene, referred to herein and HP59 (SEQ ID NO: 7).

EXAMPLE 3 - PREPARATION OF ANTIBODIES AGAINST GBS TOXIN RECEPTOR

Rabbits are immunized with the synthetic peptides shown in Table 8. A-1 mg/ml solution of peptide plus KLH in 0.01M phosphate buffer is prepared. For the first immunization, 200 µg of peptide plus KLH (200 µl) and an equal volume of Freunds complete adjuvant, emulsified well before injection, is injected into 3-4 spots on the dorsal surface about the neck and shoulders of a rabbit. After two weeks, the second immunization (boost) is given at the same concentration of immunogen, but emulsified in Freunds incomplete adjuvant. The boost is delivered in the same region of the body. After another two weeks, blood is collected and assayed by ELISA for response against the peptide without KLH. Further boosts are given to improve antibody titer, if necessary.

Table 8
Immunogenic Peptides

15

20

5

10

<u>Peptide</u>	Amino Acid Sequence	<u>Size</u>	SEQ ID Ref.
p56a	APSDGEEGSDRTPLLQRAPRAEPAPVC	27 aa	residues 8-35 of SEQ ID NO:4
p55a	LAPSDGEEGSDRTPL	15 aa	residues 7-22 of SEQ ID NO: 4
p57a	NTTAKDNRTSYECA	14 aa	residues 71-84 of SEQ ID NO: 4

Peptide p55 is a fragment of an extracellular domain of GBS toxin receptor. Peptide p57a is a fragment of an intracellular domain of GBS toxin receptor. Animals immunized with these peptides produce polyclonal antibodies Pab55 and Pab57, respectively.

EXAMPLE 4 - DETECTION OF GBS TOXIN RECEPTOR EXPRESSION IN TUMOR CELLS

This example shows that GBS toxin receptor can be detected in tumor cells.

Immunohistochemistry is performed on paired human and mouse tissues of normal or tumor origin, using rabbit polyclonal antibodies Pab 55 and Pab 57.

Mouse and human tumor tissues are fixed in 10% neutral formalin. The tissues are then dehydrated, paraffin embedded and 10-20 x 8-micron sections are cut for immunohistochemical staining.

Immunohistochemical analysis is performed with the automated Ventana Immunohistochemical Stainer according to the manufacturer's suggested protocol 5 (Ventana, Tucson, Arizona). Sections are deparaffinated with xylene. The prepared sections are then treated with 1% hydrogen peroxide prepared in 30% aqueous methanol for 20 minutes at room temperature to quench endogenous peroxidase activity. The slides are then washed with PBS, blocked with 5% BSA and 5% goat serum in PBS, washed again and then incubated for 30 minutes at 37°C with the 10 appropriate diluted (1:100) antibody. Horseradish peroxidase-labeled goat anti-rabbit IgG is used as a secondary antibody. For visualization, the sections are incubated with DAB/H₂O₂. The sections are finally incubated with a copper enhancer (Ventana) for 4 minutes, washed, counterstained with hematoxylin, and mounted in tolueneminus mounting medium. Photographic documentation is performed and images are stored for later review and analysis. The results are summarized in Table 9. The numbers refer to glass slides.

TABLE 9 Immunohistochemistry of tumor and normal tissues

(diff. = differentiated)

Human tissues:

15

	Antibody	Magnification	Signal
1. Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 55	400x	+
2. Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 55	400x	+
3. Normal ovary (96-08ZO08) control tissue	Pab 55	400x	-
4. Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 57	400x	+
5. Ovary tumor (9 5 -02VO 16) high grade papillary carcinoma	Pab 57	400x	+
6. Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 57	400x	+
7. Normal ovary 96-08ZO08) control	Pab 57	400x	
8. Colon cancer 95-14664) poorly diff. Adenocarcinoma	Pab 55	400x	+
9. Normal colon 9708VO08) control	Pab 55	400x	
10. Colon cancer 95-14664) poorly diff. Adenocarcinoma	Pab 57	400x	+

WO 00/053/5			PCT/US99/16676
11. Colon cancer 95-14664) poorly diff. Adenocarcinoma	Pab 57	400x	· +
12. Normal colon 9708VO08) control	Pab 57	400x	
13. Female breast cancer (97-IOV03a)	Pab 55	400x	+
Invasive mammary carcinoma			·
14. Male breast cancer (no code)	Pab 55	400x	+
mammary carcinoma			
15. Normal female breast 97-12VO20-3)	Pab 55	400x	-
control			
16. Female breast cancer 97-IOV03a)	Pab 57	400x	+
Invasive mammary carcinoma			
17. Male breast cancer (no code)	Pab 57	400x	+
mammary carcinoma			
18. Normal female breast (97-12VO20-3)	Pab 57	400x	-
control			
19. Lung cancer (97- 1 OV022-5) poorly	Pab 55	400x	+
diff. NOJ-small cell carcinoma			
20. Normal lung (98-0 1 VO 11) control	Pab 55		-
21. Lung cancer (97-10VO22-5) poorly	Pab 57	400x	+
diff. NOJ-small cell carcinoma			

DCT//1000/16676

Mouse Tissues:

23. Normal lung (98-0 1 VO 11) control

diff. NOJ-small cell carcinoma

22. Lung cancer (97-10VO22-5) poorly Pab 57

WO 00/05375

	Antibody	Magnification		Signal
24. Madison Lung Tumor (MLT) untreated with CM 101	Pab 55			+
25. MLT untreated with CM 101	Pab 55			+
26. Normal mouse lung	Pab 55			-
27. MLT untreated with CM 101	Pab 57		* *	+
28. Normal mouse lung	Pab 57			

Pab 57

400x

cancer tissue section, but such staining is not apparent in cells of normal human ovary tissue (see FIG. 2A and 2B, respectively). Similar results are obtained with the Pab 57 antibody (see FIG. 3A and 3B). As shown in the above table and in FIGS. 2A-3B, antibodies raised to GBS toxin receptor fragments specifically bound to tumor tissues but not normal tissues, suggesting that GBS toxin receptor is expressed in tumor cells but not normal cells.

The Pab 55 antibody stains the cells lining a blood vessel in a human ovary

10 EXAMPLE 5- DETECTION OF GBS TOXIN RECEPTOR EXPRESSION IN MICE AFFLICTED WITH RHEUMATOID ARTHRITIS

This example shows that GBS toxin receptor can be detected in cells from a mammalian model for rheumatoid arthritis (RA). Mice with collagen-induced arthritis were treated with CM101 or carrier. CM101 reversed the inflammatory

damage and inhibited pannus formation. Mouse #8 and #15, which were treated with CM101, and two control mice (not treated with CM101) were sacrificed for immunohistochemistry.

TABLE 10 Immunohistochemistry of Rheumatoid Arthritic Mice

29. No CM 101	Pab 55	+
30. MOUSE 8 - 5' (vessel)	Pab 55	+
31. No CM 101	Pab 57	+
32. MOUSE 15 - 5' (vessel)	Pab 57	+
33. MOUSE 8 - 5' (between joint)	Pab 57	+
34. MOUSE 15 – 5'	Pab 57	+
35. No CM 101 (marrow)	Pab 57	+
36. MOUSE 15 - 5' (marrow)	Pab 57	+

As shown above Pab55 and Pab57 specifically bound to pathologic neovasculature in the pannus, suggesting that GBS toxin receptor is expressed in mice afflicted with rheumatoid arthritis. No binding of CM101 was observed in the normal neovasculature in the growth plate of the joints of the arthritic mice.

EXAMPLE 6 - TARGETED DELIVERY OF A CHIMERIC COMPOUND TO TISSUES EXPRESSING GBS TOXIN RECEPTOR

This example shows the targeted delivery of a chimeric compound to tissues expressing GBS toxin. The chimeric compound is a CM101-biotin conjugate. Mice with Madison Lung Tumors (MLT) are infused intravenously (i.v.) with biotinylated CM 101.

CM101 has been reacted with hydrazinylated biotin to form the biotin

20 hydrazone at the reducing end of the polysaccharide CM101. Briefly, 25 micrograms of lyophilized CM101 is dissolved in 250 μl labeling buffer at 100 mM sodium acetate, 0.02% sodium azide. Aqueous meta-periodate (125 μl of 30 mM) is added and the oxidation is allowed to proceed in the dark for 30 minutes at room temperature. The reaction is terminated by adding 80 mM Na₂SO₃ to the solution.

25 The resultant aldehydes are reacted with 125 μl of 5 mM NHS-LC-Biotin (MW 556.58) for a 1 hour incubation at room temperature to form biotinylated CM101.

Excess biotin is removed by dialysis against 1 liter of PBS at 4°C four times. The

5

product is purified by gel filtration on an Ultrahydrogel 1000 HPLC. lyophilized and stored at -70°C until use.

Tissues are recovered 5 min post infusion with CM101 and subjected to immunohistochemistry. Tumor and normal mouse tissue sections are analyzed for CM 101 binding by both mouse anti-CM101 mAb (7A3), followed by secondary mAb-HRP conjugate (referred to in FIG. 4B as MLT CM101-Biot.5' + McAb), or with avidin (which specifically binds biotin) conjugated with HRP (referred to in FIG. 4A as MLT CM101-Biot.5' + Strep.HRP).

FIGS. 4A-4C depict different sections taken from the same tumor and include a longitudinal view of the same blood vessel approximately in the center of the figures. The dark staining in FIG. 4A shows the localization of the biotin component in the cells lining the blood vessel. Similarly, FIG. 4B depicts the localization of the CM101 component in the cells lining the blood vessel. FIG. 4C is a negative control that was not exposed to CM101. The analysis clearly shows that 7A3 and avidin bind to the same blood vessels in tumor tissue. Thus, biotin has been delivered to the blood vessel of the tumor tissue by virtue of its physical association with a compound (CM101) that binds the GBS toxin receptor.

These studies show that chimeric compounds can be delivered to tissues undergoing pathologic and/or hypoxia-driven angiogenesis or neovascularization. As part of a chimeric compound, cytotoxic molecules can be directed to such tissues, e.g., tumor tissue. The cytotoxic molecule can be coupled directly to a molecule that binds GBS toxin receptor, e.g., GBS toxin. Alternatively, the molecule that binds GBS toxin receptor can be coupled to biotin and the cytotoxic molecule can be coupled to avidin.

5

10

15

EXAMPLE 7 – ENHANCED SENSITIVITY TO GBS-TOXIN-DEPENDENT CYTOTOXICITY OF CELLS EXPRESSING GBS TOXIN RECEPTOR

This example shows the enhanced sensitivity to GBS-toxin-dependent cytotoxicity of cells transfected with the GBS toxin receptor, relative to control cells. Without being bound to a particular theory, the inventors believe that complement binds GBS toxin bound to the GBS toxin receptor on a cell, thereby targeting the cell for killing by white blood cells (WBC).

Human bladder carcinoma cells (ECV cells), are stable transfected with the human GBS toxin receptor gene. The resultant cell line is ECV711. Cells stable transfected with vector alone as referred to as V23. ECV 711 and V23 are seeded in 96-well plates at 5,000 cells/well.

White blood cells are collected from healthy human donors as follows. Blood is collected by standard phlebotomy procedures into heparinized tubes (30 U/ml) and centrifuged at 2000 rpm for 20 min. The interface is carefully transferred to a new tube and washed twice by centrifugation with medium (RPMI-1640). Cells are resuspended in RPMI-1640 supplemented with 5% fetal bovine serum (FBS) and Interferon-gamma (IFN) at 100 U/ml, and incubated overnight in a 37°C, 5%CO₂ incubator. The cells are then resuspended in fresh medium with 5% FBS.

5,000 cells of the WBC preparation are added to each well containing the transfected cells. CM101 is added to a final concentration of $1\mu g/ml$ to the wells together with human serum from matching human donors. The cells are incubated 6 hours at 37 ° C.

Cytotoxicity is assayed by measuring lactate dehydrogenase (LDH) using the Promega's CytoTox 96 Non-Radioactive Assay kit (Nachlas et al. (1960) Anal.

25 Biochem 1, 317; Korzeniewski et al. (1983) J. Immunol. Methods 64, 313; Decker et al. J. Immunol. Methods 115, 61; Brander et al. (1993) Eur. J. Immunology 23, 3217; Behl et al. (1994) Cell 77, 817; Lappalainen et al. (1994) Pharm. Research 11, 1127; Allen et al. (1994) Promega Notes 45, 7; Sinensky et al. (1995) Toxicol. Letters 75, 02; Moravec (1994) Promega Notes 45, 11). Percent cytotoxicity is calculated as recommended by the manufacturer's instructions. The results are shown in Table 11.

10

15

5

10

Table 11

Cytotoxicity	ECV 711	V 23
WBC, IFN, C3, -CM101	29.1%	27.5%
WBC, IFN, C3, +CM101	40.45%	22.46%

There is an increase in cytotoxicity of 39% when the ECV 711 cells are incubated with CM101, WBC and human serum (source of C3) compared to cells incubated without CM101. Control cells transfected with vector alone, V23, do not show a CM101 dependent increase in cytotoxicity.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

5

WHAT IS CLAIMED IS:

- 1. An isolated polynucleotide at least 10 bases in length comprising a nucleic acid sequence encoding, or complementary to a nucleic acid sequence encoding, a mammalian receptor for group B β-hemolytic Streptococcus toxin (GBS toxin receptor), or a polypeptide fragment thereof.
- 2. The polynucleotide of Claim 1, wherein the nucleic acid sequence comprises SEQ ID NO: 9.
- 3. The polynucleotide of Claim 1, wherein the nucleic acid sequence has 100% identity to a nucleic acid sequence selected from the group consisting of residues 61 to 1542 of SEQ ID NO: 1, residues 266 to 1870 of SEQ ID NO:7, and residues 87 to 1568 of SEQ ID NO: 3.
 - 4. The polynucleotide of Claim 1, wherein the polynucleotide is hybridizable under high stringency conditions to the nucleic acid sequence of SEQ ID NO: 7.
 - 5. A vector comprising the polynucleotide of Claim 1.
- 15 6. A host cell transformed with the vector of Claim 5.
 - 7. A process for producing a mammalian GBS toxin receptor or fragment thereof, comprising culturing the host cell of Claim 6 in a suitable culture medium.
 - 8. An isolated polypeptide comprising a mammalian GBS toxin receptor or fragment thereof.
- 9. The polypeptide of Claim 8, wherein the receptor has at least about 86% identity to the corresponding amino acid sequence of SEQ ID NO: 2.
 - 10. The polypeptide of Claim 8, wherein the receptor or fragment has 100% identity to the corresponding region of the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 8.
- 25 11. The polypeptide of Claim 8, wherein the polypeptide is encoded by a nucleic acid sequence hybridizable under high stringency conditions to a nucleic acid sequence selected from the group consisting of:

- a) nucleotides 61 to 1542 of SEQ ID NO: 1, and
- b) nucleotides 87 to 1568 of SEQ ID NO: 3.
- 12. An isolated polypeptide comprising an amino acid sequence that differs from an amino acid sequence selected from the group consisting of SEQ ID NO:2,
- 5 SEQ ID NO:4 and SEQ ID NO:8 at no more than about 20% of the amino acid residues.
 - 13. The isolated polypeptide of Claim 12, wherein the amino acid sequence of said isolated polypeptide differs from the amino acid sequence selected from said group by one amino acid residue.
- 14. The isolated polypeptide of Claim 12, wherein the different amino acid residues are conservative substitutions of the corresponding residues of the amino acid sequence selected from said group.
 - 15. An isolated polypeptide comprising an amino acid sequence of the formula:

15

20

25

AA1-AAn-AAm

wherein:

AA1 is absent or is M:

AAn is a contiguous chain of 0 to 100 amino acids, preferably of 0 or 41 amino acids, even more preferably of residues 2-42 of SEQ ID NO:8; and

AAm is a contiguous chain of 494 amino acids comprising AA43 through AA536, wherein:

(1) each of AA43, AA47, AA51, AA52, AA57, AA58, AA65, AA66, AA72, AA85, AA87, AA93, AA94, AA96, AA115, AA116, AA122, AA123, AA125, AA134, AA143, AA173, AA174, AA178, AA185, AA186, AA189, AA190, AA196, AA200, AA204, AA206, AA207, AA220, AA253, AA260, AA276, AA277, AA280, AA283, AA287, AA294, AA295, AA298, AA300, AA301, AA312, AA324, AA326, AA360, AA365, AA373, AA374, AA379, AA396, AA403, AA407, AA418, AA480, AA483, AA486, AA491, AA494, AA502, AA528, AA529, AA532 and AA536 is an amino acid residue corresponding to:

30 (a) residue 43, 47, 51, 52, 57, 58, 65, 66, 72, 85, 87, 93, 94, 96, 115, 116, 122, 123, 125, 134, 143, 173, 174, 178, 185, 186, 189, 190, 196, 200, 204, 206, 207, 220, 253, 260, 276, 277, 280, 283, 287, 294, 295, 298, 300, 301, 312, 324, 326, 360, 365, 373, 374, 379, 396, 403, 407, 418, 480, 483, 486, 491, 494, 502, 528, 529, 532 and 536, respectively, of SEQ ID NO:8;

35 (b) residue 2, 6, 10, 11, 16, 17, 24, 25, 31, 44, 46, 52, 53, 55, 74, 75, 81, 82, 84, 93, 102, 132, 133, 137, 144, 145, 148, 149, 155, 159, 163, 165, 166, 179, 212, 219, 235, 236, 239, 242, 246, 253, 254, 257, 259, 260, 271, 283,

285, 319, 324, 332, 333, 338, 355, 362, 366, 377, 439, 442, 445, 450, 453, 461, 487, 488, 491 and 495, respectively of SEQ ID NO:4; or

(c) a conservative substitution thereof;

- (2) each of AA44-AA46, AA48-AA50, AA53-AA56. AA59-AA64, AA67-AA71, AA73-AA84, AA86, AA88-AA92, AA95, AA97-AA114, AA117-AA121, AA124, AA126-AA133, AA135-AA142, AA144-AA172, AA175-AA177, AA179-AA184, AA187-AA188, AA191-AA195, AA197-AA199, AA201-AA203, AA205, AA208-AA219, AA221-AA252, AA254-AA259, AA261-AA275, AA278-AA279, AA281-AA282, AA284-AA286, AA288-AA293, AA296-AA297, AA299,
- 10 AA302-AA311, AA313-AA323, AA325, AA327-AA359, AA361-AA364, AA366-AA372, AA375-AA378, AA380-AA395, AA397-AA402, AA404-AA406, AA408-AA417, AA419-AA478, AA481-AA482, AA484-AA485, AA487-AA490, AA492-AA493, AA495-AA501, AA503-AA527, AA530-AA531 and AA533-AA535 is

 (a) residue 44-46, 48-50, 53-56, 59-64, 67-71, 73-84,
- 15 86, 88-92, 95, 97-114, 117-121, 124, 126-133, 135-142, 144-172, 175-177, 179-184, 187-188, 191-195, 197-199, 201-203, 205, 208-219, 221-252, 254-259, 261-275, 278-279, 281-282, 284-286, 288-293, 296-297, 299, 302-311, 313-323, 325, 327-359, 361-364, 366-372, 375-378, 380-395, 397-402, 404-406, 408-417, 419-478, 481-482, 484-485, 487-490, 492-493, 495-501, 503-527, 530-531 and 533-535, respectively, of SEQ ID NO:8; or
 - (b) a conservative substitutions thereof; and (3) one or more of AA315 through AA367 are optionally absent.
 - 16. An antibody that recognizes a mammalian GBS toxin receptor or fragment thereof.
- 25 17. An isolated complex comprising a GBS toxin bound to a mammalian GBS toxin receptor or fragment thereof.
 - 18. A method of forming a complex comprising:

contacting a GBS toxin with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that permit specific binding of the GBS toxin to the polypeptide, and allowing the complex to form.

19. A method for purifying a compound that binds a GBS toxin receptor, which method comprises:

providing a polypeptide comprising a mammalian GBS toxin receptor or fragment thereof that binds GBS toxin;

contacting said polypeptide with a sample comprising the compound under conditions that allow specific binding of the compound to the polypeptide; and

separating the bound compound from the remainder of the sample.

20. A method of determining the presence or absence of GBS toxin in a sample, which method comprises:

contacting the sample with a polypeptide comprising a mammalian

GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that permit specific binding of the GBS toxin to the polypeptide,

and determining whether specific binding has occurred.

- 21. A method for diagnosing early onset disease in a neonate comprising performing the method of Claim 20, wherein the sample is obtained from the neonate and wherein presence of the GBS toxin is indicative of early onset disease.
- 22. A method for detecting pathologic vasculature in a mammalian tissue, which method comprises detecting the presence of a GBS toxin receptor.
- 23. A method for identifying a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor, comprising:
- 15 combining a test compound with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, in a reaction mixture containing GBS toxin and under conditions that permit specific binding of the GBS toxin to the receptor or fragment, and
- determining the amount of inhibition by the compound of the binding of the GBS toxin to the polypeptide.
 - 24. An inhibitor of binding of a GBS toxin to a mammalian GBS toxin receptor.
 - 25. A method for identifying a compound which specifically binds a mammalian GBS toxin receptor, comprising:
- combining a test compound with a polypeptide comprising a mammalian GBS toxin receptor or fragment thereof that can bind GBS toxin, under conditions that allow specific binding to occur, and

detecting a complex formed between said test compound and said

polypeptide.

26. A method for determining cytotoxicity of a test chimeric compound, which method comprises:

exposing a cell expressing, on the cell surface, a mammalian GBS

toxin receptor, or fragment thereof that binds GBS toxin, to a test chimeric compound comprising a cytotoxic agent coupled to said GBS toxin; and detecting signs of cytotoxicity.

- 27. A chimeric compound comprising a cytotoxic agent covalently linked to a molecule that specifically binds a mammalian GBS toxin receptor.
- 10 28. A method for identifying an inhibitor of GBS toxin receptor, which method comprises:

incubating test cells in the presence and absence of a test compound and under conditions in which the cells incubated in the absence of the test compound can proliferate or migrate, wherein the test cells express GBS toxin receptor or a fragment thereof having GBS toxin receptor activity; and

comparing the proliferation or migration of the test cells incubated in the presence of the test compound to the proliferation or migration of the test cells incubated in the absence of the test compound, wherein less proliferation or migration in the presence of the test compound is indicative of the test compound being an inhibitor of the GBS toxin receptor.

29. A method for identifying an inhibitor of endothelial cell proliferation or migration, which method comprises:

incubating test endothelial cells in the presence and absence of a test compound and under conditions in which the cells incubated in the absence of the test compound can proliferate or migrate, wherein the test cells express GBS toxin receptor or a fragment thereof having GBS toxin receptor activity; and

comparing the proliferation or migration of the test cells incubated in the presence of the test compound to the proliferation or migration of the test cells incubated in the absence of the test compound, wherein less proliferation or migration

15

20

in the presence of the test compound is indicative of the test compound being an inhibitor of the endothelial cell proliferation or migration.

30. A method for identifying a therapeutic compound for the treatment or prevention of a medical condition characterized by pathologic angiogenesis or neovascularization, which method comprises:

incubating test cells in the presence and absence of a test compound, wherein the test cells express GBS toxin receptor or a fragment thereof having GBS toxin receptor activity;

the presence of the test compound to the proliferation or migration of the test cells incubated in the presence of the test compound to the proliferation or migration of the test cells incubated in the absence of the test compound, wherein less proliferation or migration in the presence of the test compound is indicative of the test compound being a candidate therapeutic compound for the treatment or prevention of the medical condition.

- 31. The method of Claim 30, wherein the medical condition is a cancerous tumor.
 - 32. The method of Claim 30, wherein the medical condition is a reperfusion injury.
- 33. The method of Claim 30, wherein the medical condition is scarring during wound healing.
 - 34. The method of Claim 30, wherein the medical condition is keloids.
 - 35. The method of Claim 30, wherein the medical condition is a chronic inflammatory disease.
 - 36. The method of Claim 30, wherein the medical condition is neural injury.
- 25 37. A method for identifying a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor, comprising:
 - (a) simulating and selecting the most probable conformations of a mammalian GBS toxin receptor,

5

(b) designing a chemically modified analog that substantially mimics the energetically most probable three-dimensional structure of the polypeptide,

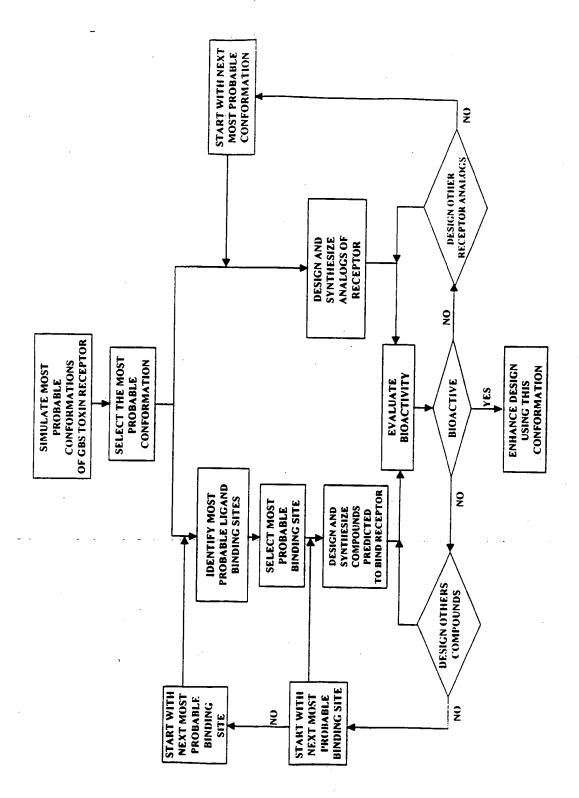
- (c) chemically synthesizing the analog, and
- (d) evaluating the bioactivity of the analog.
- 5 38. A method for identifying a compound which binds to a mammalian GBS toxin receptor, comprising:
 - (a) simulating and selecting the most probable conformations of a mammalian GBS toxin receptor,
 - (b) deducing the most probable binding domains of the polypeptide,
- (c) designing a compound that would form the energetically most probable complexes with the polypeptide,
 - (d) chemically synthesizing the compound, and
 - (e) evaluating the bioactivity of the compound.
- 39. A method for the prevention or treatment of neonatal onset disease in a
 human neonate, comprising administering an inhibitor of binding of GBS toxin to a
 human GBS toxin receptor.
- 40. A method for inhibiting pathologic or hypoxia-driven endothelial cell proliferation or migration in a mammalian tissue, which method comprises specifically binding a molecule to a GBS toxin receptor present on the surface of at
 least one cell in the tissue, the molecule being selected from the group consisting of:
 - a compound that can evoke an inflammatory response when bound to a GBS toxin receptor in a mammal;
 - a chimeric compound comprising a cytotoxic compound coupled to a compound that specifically binds the GBS toxin receptor;
- an inhibitor of GBS toxin receptor phosphorylation; and an inhibitor of GBS toxin receptor activity.
 - 41. A pharmaceutical composition comprising a pharmaceutically effective amount of a molecule selected from the group consisting of:
 - a GBS toxin receptor or fragment thereof;

an inhibitor of a GBS toxin receptor; and

a chimeric compound comprising a cytotoxic agent coupled to a compound that binds GBS toxin receptor, and a pharmaceutically acceptable carrier.

5

- 42. A kit comprising a component selected from the group consisting of:
 - a GBS toxin receptor or fragment;
- a reagent for detecting the presence of a GBS toxin receptor or fragment; and
- a reagent for detecting the presence of polynucleotide encoding the GBS toxin receptor or fragment.
 - 43. A molecule for use in a method of treatment of the human or animal body, said molecule being selected from the group consisting of:
- a GBS toxin receptor or fragment thereof for use in a method of treatment of the human or animal body, said molecule being selected from the group consisting of:
 - a GBS toxin receptor or fragment thereof; an inhibitor of binding of GBS toxin to a GBS toxin receptor; an inhibitor of a GBS toxin receptor; and
 - a chimeric compound comprising a cytototoxic agent coupled to a compound that binds GBS toxin receptor.
- 44. Use of an inhibitor of a GBS toxin receptor, or of an inhibitor of binding of GBS toxin to a GBS toxin receptor, for the manufacture of a medicament for the treatment of a medical condition characterized by pathologic or hypoxia-driven angiogenesis or neovascularization.

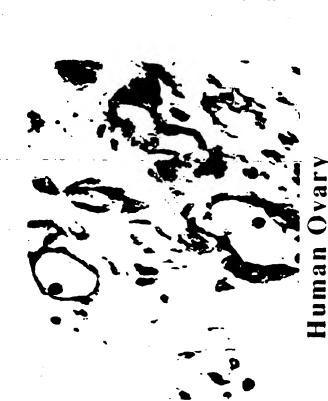


יוני ז









Normal Human Ovary+Pab 57

FIG



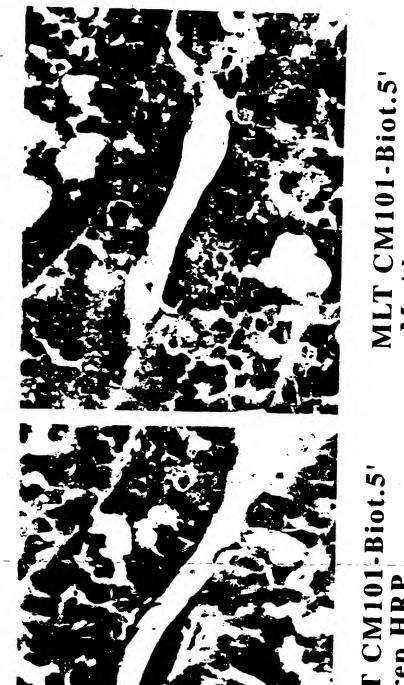
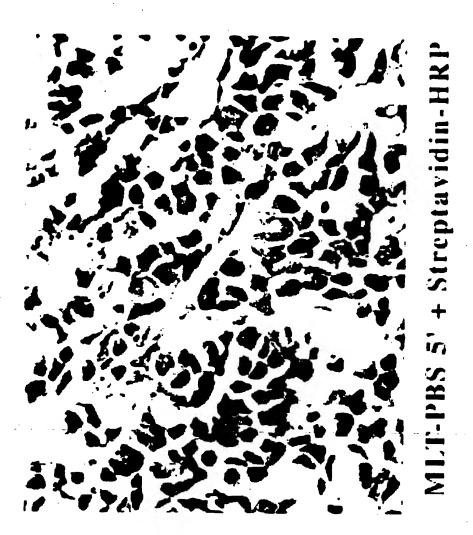


FIG. 4A





SEQUENCE LISTING

	<110>	Hell	erqv	ist,	Car	1										
		Fu,	Chan	glin												
	_					,										
	<120>	GBS	Toxi	n Re	cept	or										
															•	
	<130>	CARB	-008	/01W	0											
	<140>															
	<141>															
	<150>	60-0	93 A	4 7												
	<151>															
	\ 1 J1>	1990	-0,-2	22												
	-160-	• •														
	<160>	12														
	<170>	Pater	ntIn	Ver.	. 2.0)										
	<210>	_														
	<211>	2602														
	<212>	DNA														
	<213>	Homo	sapi	.ens												
	<220>															
	<221>	CDS														
	<222>	(58).	. (15	42)												
	<400>	1														
	tcgggc	cggc	gctc	cctt	ct c	tgcc	aggt	g gc	gagt	acac	ctg	ctca	cgt	aggc	gtc	57
															_	
	atg ag	g tct	ccg	gtt	cga	gac	ctg	gcc	cgg	aac	gat	ggc	gag	gag	age	105
	Met Ar															
	1			5	_	-			10		-	٠.		15		
-		8 -		-; -						-	-					
	acg ga	c cqc	acq	cct	ctt	cta	cca	aac	acc	cca	caa	acc	даа	acc	act	153
	Thr As															
			20					25			5		30		,,,,,	
			. 7										30			
	cca gt	t tac	tac	tet	act	cat	t ac	220	++=	~~~						202
												_	_			201
	Pro Va	-	-	261	Ala	ALG	-	ASII	Leu	WIG	116		ALA	Pne	Pne	
		35					40					45				
				-6												
	ggt tt										-	_	-			249
	Gly Pho		Ile	Val	Tyr		Leu	Arg	Val	Asn		Ser	Val	Ala	Leu	
	5)			-	55					60					
	gtg ga	atg	gta	gat	tca	aat	aca	act	tta	gaa	gat	aat	aga	act	tcc	297

Val 65) Met	: Va]	l Asp	Se: 7		n Thi	r Thi	Lei	u Gli 79		Ası	ı Arg	g Thi	Ser 80	
					His					E Lys					caa Gln	345
				Туг					Glu					Ile	ctc Leu	393
			Phe										Gly		tat Tyr	441
							Lys	atg Met								489
								act Thr								537
								gca Ala								585
								atg Met 185								633
								att Ile								681
								tct Ser		Ile		tgc Cys			atg Met	729
								ttt Phe						Phe		777
			Trp					agt Ser					Lys		_	825
aga	att	tcc	cat	tat	gaa	aag	gaa	tac	att	ctt	tca	tca	tta	aga	aat	873

Arg	Ile	Ser	260		Gli	ı Lys	5 Gl:	265		e Le	u Sei	r Sei	270		g Asn	
			Ser					l Pro					Leu		a tcc S Ser	921
		Leu					. Val					Tyr			act Thr	969
						Leu					Met				cta Leu 320	101
										Ser		_			tta Leu	106
													-		tta Leu	111:
				aat Asn					-	_	_	_			_	1161
				att Ile			_	_			_	-	_			1209
				tat Tyr		_	_	-	_							1257
				ttt Phe 405	_					_				_	_	1305
				tat Tyr								Thr				1353
				gga Gly	_				-		-		_	_		1401
																

Pro Asp Asn Thr Val Gly Glu Trp Gln Thr Val Phe Tyr Ile Ala Ala 450 455 460	
gct att aat gtt ttt ggt gcc att ttc ttt aca cta ttc gcc aaa ggt Ala Ile Asn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly 470 475 480	149
gaa gta caa aac tgg gct ctc aat gat cac cat gga cac aga cac Glu Val Gln Asn Trp Ala Leu Asn Asp His His Gly His Arg His 485 490 495	1542
tgaaggaacc aataaataat cctgcctcta ttaatgtatt tttatttatc atgtaacctc	
aaagtgcctt ctgtattgtg taagcattct atgtcttttt ttaattgtac ttgtattaga	
tttttaaggc ctataatcat gaaatatcac tagttgccag aataataaaa tgaactgtgt	
ttaattatga ataatatgta agctaggact tctactttag gttcacatac ctgcctgcta	1782
gtcgggcaac atgaagtagg acagttctgt tgatttttta gggccatact aaagggaatg	1842
agetgaaaca gaceteetga tacetttget taattaaact agatgataat teteaggtae	1902
tgataaacac ctgttgttgt tcactttcct cataaaaatt gtcagctctc tctgacactt	1962
agaceteaaa etttageate tetgtggage tgecatecae tgtataattt egeetggeaa 2	2022
ctggactgag gggagtgtgc ccaggcagct gccaagcact ccctccctgg cttcagggtc 2	082
agagtgccca gegtttatca gaggcagcat ccaagcccag agccagtgte gactettegg 2	142
ctggtgcctt tectetgagg ggetatcaat gtgtagataa ageeetgagt aggeaagage 2	202
agtgagatec actgetatgg tettgataca teetcaaact tteeetteec ageacagagg 2	262
aatattgget ggcatgcaac ctgcaaaaga aaaatgcgaa gcggccgggc acggtggetc 2	322
atgeetgtaa teecageaet ttgggggget gaggtgggeg aateatgaga teaggagtte 2	382
gagaccagee tggeeageat ggtgaaaeee catetetaet aaaaataeaa aaaattaget 2	142
gggcgtggtg acgggcgcct gtaatcccag atactcagga ggctgaggta ggagaatcac 2:	502
tgaacctgg gaggtggaag ttgcagtgaa ccaagatcac gccactgcac tccagcctgg 25	i 6 2
cgatggagc gagactccaa ctcaaaaaaaa aaaaaaaaaa	02

<210> 2

<211> 495

<212> PRT

<213> Homo sapiens

<400> 2

Met Arg Ser Pro Val Arg Asp Leu Ala Arg Asn Asp Gly Glu Glu Ser 1 5 10 15

Thr Asp Arg Thr Pro Leu Leu Pro Gly Ala Pro Arg Ala Glu Ala Ala
20 25 30

Pro Val Cys Cys Ser Ala Arg Tyr Asn Leu Ala Ile Leu Ala Phe Phe 35 40 45

Gly Phe Phe Ile Val Tyr Ala Leu Arg Val Asn Leu Ser Val Ala Leu 50 55 60

Val Asp Met Val Asp Ser Asn Thr Thr Leu Glu Asp Asn Arg Thr Ser 65 70 75 80

Lys Ala Cys Pro Glu His Ser Ala Pro Ile Lys Val His His Asn Gln 85 90 95

Thr Gly Lys Lys Tyr Gln Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu 100 105 110

Gly Ser Phe Phe Tyr Gly Tyr Ile Ile Thr Gln Ile Pro Gly Gly Tyr 115 120 125

Val Ala Ser Lys Ile Gly Gly Lys Met Leu Leu Gly Phe Gly Ile Leu 130 135 140

Gly Thr Ala Val Leu Thr Leu Phe Thr Pro Ile Ala Ala Asp Leu Gly
145 150 155 160

Val Gly Pro Leu Ile Val Leu Arg Ala Leu Glu Gly Leu Gly Glu Gly 165 170 175

Val Thr Phe Pro Ala Met His Ala Met Trp Ser Ser Trp Ala Pro Pro 180 185 190

Leu Glu Arg Ser Lys Leu Leu Ser Ile Ser Tyr Ala Gly Ala Gln Leu 195 200 205

Gly Thr Val Ile Ser Leu Pro Leu Ser Gly Ile Ile Cys Tyr Tyr Met 210 215 220

Asn Trp Thr Tyr Val Phe Tyr Phe Phe Gly Thr Ile Gly Ile Phe Trp
225 230 235 240

- Phe Leu Leu Trp Ile Trp Leu Val Ser Asp Thr Pro Gln Lys His Lys
 245 250 255
- Arg Ile Ser His Tyr Glu Lys Glu Tyr Ile Leu Ser Ser Leu Arg Asn 260 265 270
- Gln Leu Ser Ser Gln Lys Ser Val Pro Trp Val Pro Ile Leu Lys Ser 275 280 285
- Leu Pro Leu Trp Ala Ile Val Val Ala His Phe Ser Tyr Asn Trp Thr 290 295 300
- Phe Tyr Thr Leu Leu Thr Leu Leu Pro Thr Tyr Met Lys Glu Ile Leu 305 310 315 320
- Arg Phe Asn Val Gln Glu Asn Gly Phe Leu Ser Ser Leu Pro Tyr Leu 325 330 335
- Gly Ser Trp Leu Cys Met Ile Leu Ser Gly Gln Ala Ala Asp Asn Leu 340 345 350
- Arg Ala Lys Trp Asn Phe Ser Thr Leu Cys Val Arg Arg Ile Phe Ser 355 360 365
- Leu Ile Gly Met Ile Gly Pro Ala Val Phe Leu Val Ala Ala Gly Phe 370 380
- Ile Gly Cys Asp Tyr Ser Leu Ala Val Ala Phe Leu Thr Ile Ser Thr 385 390 395 400
- Thr Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Asp
- Ile Ala Pro Ser Tyr Ala Gly Ile Leu Leu Gly Ile Thr Asn Thr Phe
 420 425 430
- Ala Thr Ile Pro Gly Met Val Gly Pro Val Ile Ala Lys Ser Leu Thr 435 440 445
- Pro Asp Asn Thr Val Gly Glu Trp Gln Thr Val Phe Tyr Ile Ala Ala 450 455 460
- Ala Ile Asn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly
 465 470 475 480

Glu Val Gln Asn Trp Ala Leu Asn Asp His His Gly His Arg His
485 490 495

<210> 3

<211> 2844

<212> DNA

<213> Ovis sp.

<220>

<221> CDS

<222> (84) .. (1568)

<400> 3

cccgggggcg gggggcttcg gcggtcccgc tggagctctc ttttccgcgg agcaggtttg 60

cgccgtagct ccctgaaggc atc atg aag tcc ccg gtt tcg gac tta gcc ccg 113

Met Lys Ser Pro Val Ser Asp Leu Ala Pro

1 5

age gae gge gag gge teg gae ege aca eeg ete etg eag ege gee 161 Ser Asp Gly Glu Glu Gly Ser Asp Arg Thr Pro Leu Leu Gln Arg Ala 15 20 25

ccg cgg gcg gaa ccc gct cca gta tgc tgc tct gct cgt tac aac cta 209
Pro Arg Ala Glu Pro Ala Pro Val Cys Cys Ser Ala Arg Tyr Asn Leu
30 35 40

gca ttt ttg tcc ttt ttt ggt ttc ttc gtt ctc tat tca tta cgg gtg
Ala Phe Leu Ser Phe Phe Gly Phe Phe Val Leu Tyr Ser Leu Arg Val
45 50 55

aat ctg agc gtt gca cta gtg gac atg gtg gat tca aac aca act gcc 305
Asn Leu Ser Val Ala Leu Val Asp Met Val Asp Ser Asn Thr Thr Ala
60 65 70

aaa gat aat aga acg tcc tac gag tgt gca gag cat tct gct ccc ata 353
Lys Asp Asn Arg Thr Ser Tyr Glu Cys Ala Glu His Ser Ala Pro Ile
75 80 85

aaa gtt ctt cac aac caa acg ggt aaa aag tac cgg tgg gat gca gaa 403 Lys Val Leu His Asn Gln Thr Gly Lys Lys Tyr Arg Trp Asp Ala Glu 95 . 100 105

act caa gga tgg att ctc gga tct ttt ttc tat ggc tac atc atc aca 449
Thr Gln Gly Trp Ile Leu Gly Ser Phe Phe Tyr Gly Tyr Ile Ile Thr
110 115 120

															J ttg Leu	497
		125	;				130)				135	•			
															ccc	545
Leu	140		e GIÀ	/ 11e	e Pne	145		Alā	i Ile	e Phe	150		Phe	e Thi	Pro	
															cta	593
155	Ala	. Ala	Asp) Pne	160		. Gly	' Ala	ı Leı	1 Val		. Leu	Arg	, Ala	170	
												cat				641
Glu	Gly	Leu	Gly	Glu 175		Val	Thr	Tyr	180		Met	His	Ala	Met 185	•	
												ctg				689
			190		٠			195				Leu	200			
												cct				737
		205					210	÷				Pro 215			•	
												tat Tyr				785
	220					225		٠			230				•	
												tta				833
235					240					245		Leu			250	
												aag				881
īūr	PIO	ĠΤĦ	THE	255	rÀä	Tür	īīe	īņr	260	ıyr	Gin	Lys	GIu	265	Ile	
												tca		_		929
Leu	ser	ser	270	Lys	Asn	Gln	Leu	Ser 275	Ser	Gln	Lys	Ser	Val 280	Pro	Trp	
												gtc	-	_		977
116	rio	мес 285	ren	ъуs	ser	ьeu	Pro 290	ren	ırp	AIA		Val ' 295	val	Ala	His	
									_			tta 1	_			1025
	ser 300	ıyr	Asn	Trp		Phe 305	Tyr	Thr	ren		Thr 310	Leu 1	Leu	Pro	Thr	
						~										

	Met					Arg					ı Glu				t tta e Leu 330	1073
					Leu										ggt Gly	1121
				Asn										Leu	tgg Trp	1169
															ttc Phe	1217
		Ala							gat Asp							1265
Phe 395	Leu	Thr	Ile	Ser	Thr 400	Thr	Leu	Gly	ggc	Phe 405	Суз	Ser	Ser	Gly	Phe 410	1313
Ser	Ile	Asn	His	Leu 415	Asp	Ile	Ala	Pro	tcg Ser 420	Tyr	Ala	Gly	Ile	Leu 425	Leu	1361
Gly	Ile	Thr	Asn 430	Thr	Phe	Ala	Thr	Ile 435	cct Pro	Gly	Met	Ile	Gly 440	Pro	Ile	1409
Ile	Ala	Arg 445	Ser	Leu	Thr	Pro	Glu 450	Asn	act Thr	Ile	Gly	Glu ' 455	Trp	Gln	Thr	1457
Val	Phe 460	Cys	Ile	Ala	Ala i	Ala : 465	Ile .	Asn '	Val	Phe	Gly 470	Ala :	Ile	Phe		1505
Thr 475	Leu	Phe	Ala	Lys	Gly (480	3lu 1	Val (Gln :		Trp . 485	Ala	Ile s	Ser i	Asp	His 49 0	1553
		cac His	Arg .		tgaaq	gaad	ec a	ataa	ataa	t cc	tgtc	tcta	ttaa	atgt.	at c	1608

tttgtttatc atgtaaccta aaagtgcctt tgatatttta atgtgtaagc aatctatata 1668 caaqataaaa ttqtactaqa aaaattqtqt tagatttgta aggcttqtaa tcatqaaatq 1728 tcactagttg ccatataagc aaaattagct atttttaatt attattaacc cgtttgctgg 1788 aacttacaat tcagggtcac atatctggct gcaagtcagg caacccacaa taggggagtt 1848 ctatttattt ataagaccat acctaaagag atgagctgaa atagaccett ctataccttt 1908 qcttaattaa ggtggataat aattctcagg tcttgttaaa catctgtttt tgtacacctt 1968 cctcaaaaaa ttatttgtca tcagcaatcc ctgacatgta ggtctcaaac tttagcctct 2028 ccacggaget ggcagecact gtateattea geetggcaae tteactgagg gaageatgee 2088 caqqcaqctq ccacatgtcc cctctctggc ttcagggaca gtgcccagca cttaqqcaqc 2148 atccaaqacc agggtcagcg ccaaggettt ggacggtatt cttcccctgg ggctgttaat 2208 gtgtggatga agccctgagc caacagggac agcgcgatcc acagtcatgg tttccatgca 2268 contenent tecetteeca quacactgga gtattgcetg gcatgtaacc tgcaaaagaa 2328 aqtqtqatqc ctaattagcc acatataaca tcatccttga tgatcctacc ttcacatgga 2388 tcaqaqtata aatcttcaag tcctgtgttc taggagctac accagaataa ttaaaatata 2448 aaaagaaaca aaacattttt totgtotgac acctaagtgt otggttgcag ttcaaggtta 2508 aggtgacttc tacttcacat aacctgcaac cggtggtgta atcatcttta gtgttggttt 2568 cttaaatctt atttttccag tttttcctgg accatcttcc agtggttttg agcatgcttt 2628 gagggcattt atgtgattta gaacttgatt aatgtttcac tgtgtatgtt caacactacc 2688 totaatattt taactaaagc tatttaatgt aatatgatgt gtatacattc tgtaaattaa 2748 tttttaaatc tgtaaatagc tttaagttgc tatggtgata tttcttttac aaatcaaaat 2808 aaatcttttt ggaatgataa aaaaaaaaaa aaaaaa 2844

<210> 4

<211> 495

<212> PRT

<213> Ovis sp.

<400> 4

Met Lys Ser Pro Val Ser Asp Leu Ala Pro Ser Asp Gly Glu Gly 1 5 10 15

Ser Asp Arg Thr Pro Leu Leu Gln Arg Ala Pro Arg Ala Glu Pro Ala 20 25 30

Pro Val Cys Cys Ser Ala Arg Tyr Asn Leu Ala Phe Leu Ser Phe Phe 35 40 45

Gly Phe Phe Val Leu Tyr Ser Leu Arg Val Asn Leu Ser Val Ala Leu 50 55 60

Val Asp Met Val Asp Ser Asn Thr Thr Ala Lys Asp Asn Arg Thr Ser 65 70 75 80

Tyr Glu Cys Ala Glu His Ser Ala Pro Ile Lys Val Leu His Asn Gln 85 90 95

Thr Gly Lys Lys Tyr Arg Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu 100 105 110

Gly Ser Phe Phe Tyr Gly Tyr Ile Ile Thr Gln Ile Pro Gly Gly Tyr
115 120 125

Val Ala Ser Arg Ser Gly Gly Lys Leu Leu Leu Gly Phe Gly Ile Phe 130 135 140

Ala Thr Ala Ile Phe Thr Leu Phe Thr Pro Leu Ala Ala Asp Phe Gly
145 150 155 160

Val Gly Ala Leu Val Ala Leu Arg Ala Leu Glu Gly Leu Gly Glu Gly 165 170 175

Val Thr Tyr Pro Ala Met His Ala Met Trp Ser Ser Trp Ala Pro Pro 180 185 190

Leu Glu Arg Ser Lys Leu Leu Ser Ile Ser Tyr Ala Gly Ala Gln Leu 195 200 205

Gly Thr Val Val Ser Leu Pro Leu Ser Gly Val Ile Cys Tyr Tyr Met 210 215 220

Asn Trp Thr Tyr Val Phe Tyr Phe Phe Gly Ile Val Gly Ile Ile Trp
225 230 235 240

Phe Ile Leu Trp Ile Cys Leu Val Ser Asp Thr Pro Glu Thr His Lys 245 250 255

Thr Ile Thr Pro Tyr Glu Lys Glu Tyr Ile Leu Ser Ser Leu Lys Asn 260 265 270

- Gln Leu Ser Ser Gln Lys Ser Val Pro Trp Ile Pro Met Leu Lys Ser 275 280 285
- Leu Pro Leu Trp Ala Ile Val Val Ala His Phe Ser Tyr Asn Trp Thr 290 295 300
- Phe Tyr Thr Leu Leu Thr Leu Leu Pro Thr Tyr Met Lys Glu Val Leu 305 310 315 320
- Arg Phe Asn Ile Gln Glu Asn Gly Phe Leu Ser Ala Val Pro Tyr Leu 325 330 335
- Gly Cys Trp Leu Cys Met Ile Leu Ser Gly Gln Ala Ala Asp Asn Leu 340 345 350
- Arg Ala Arg Trp Asn Phe Ser Thr Leu Trp Val Arg Arg Val Phe Ser 355 360 365
- Leu Ile Gly Met Ile Gly Pro Ala Ile Phe Leu Val Ala Ala Gly Phe 370 380
- Ile Gly Cys Asp Tyr Ser Leu Ala Val Ala Phe Leu Thr Ile Ser Thr 385 390 395 400
- Thr Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Asp
 405 410 415
- Ile Ala Pro Ser Tyr Ala Gly Ile Leu Leu Gly Ile Thr Asn Thr Phe
 420 425 430
- Ala Thr Ile Pro Gly Met Ile Gly Pro Ile Ile Ala Arg Ser Leu Thr-435 440 445
- Pro Glu Asn Thr Ile Gly Glu Trp Gln Thr Val Phe Cys Ile Ala Ala 450 455 460
- Ala Ile Asn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly
 465 470 475 480
- Glu Val Gln Asn Trp Ala Ile Ser Asp His Gln Gly His Arg Asn 485 490 495

<210> 5

<211> 31 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: PCR primer <400> 5 cgggatcccg ccngcnatgc ayrshrtstg g 31 <210> 6 <211> 29 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: PCR primer ggaattccdg gdgcratktc narrtrrtt 29 <210> 7 <211> 2930 <212> DNA <213> Homo sapiens <220> <221> CDS <222> (263)..(1870) <400> 7 gtteggtega agecetecce ttaattatgt gcaattcaag teeccactge eegecegeaa 60

gcccccactc atcctcgctg cgggcagggt ggcccctgca ctttacaagg gggtgcagga 120

gcgggagacg gtcgtccgaa caccggctcc ccggcatgcg tagaccggcg ggcggagcgg 180

geteactitg egecaateet acgagaacte ecagaactee getteectag tecaacceaa 240

gccagagttg cccacaccta ag atg gcg gcg gcg atg aca ccg ccc cgc 292 Met Ala Ala Gly Ala Met Thr Pro Pro Arg 1

ccg gtc cag cca gct cgg ccc ggg ggc ttc ggg ctg tcg ggc cgg cgc Pro Val Gln Pro Ala Arg Pro Gly Gly Phe Gly Leu Ser Gly Arg Arg 20

		'		cgo		- 9 9	cg 9	aca	agt	aca	a cc	t go	t ca	ac gr	ta q	gc	qtc	ato	388
Se	r L	eu 1	Leu	Cys	5 G)	ln V	al A	Ala	Ser	Thi	Pr	o Al	а Ні	is Va	al G	114	V-1	Man	300
				3 ()					35		_			0		vai	mec	
	_									٠.	•					40			
a ~								•											
ay	9 10	בנ כ	cg	gtt	. cg	ja g	ac c	:tg	gcc	cgg	aa	: ga	t gg	c ga	g g	ag	agc	acq	436
Ar	g Se	er I	Pro	Val	. Ar	g A	sp L	eu .	Ala	Arg	Ası	As	p Gl	y Gl	u G	lu	Ser	Thr	
			45						50			•	_		5			****	
														,	5				
σa	c cc	ic a	ca	cct	CF	+													
20		, c c	·	D	· • •		.a. c	cg g	ggc	gcc	cca	cg	g gc	c ga	a go	CC (gct	cca	484
AS,	ρAI	9 1	nr	Pro	re	u Le	u P	ro (31y	Ala	Pro	Arg	g Al	a Gl	u Al	la i	Ala	Pro	
	6	0						65					7	0					
gt	g tg	c t	gc	tct	gc	t cq	t ta	ac a	ac	tta	aca	att	- ++	g gc					
Va:	l Cy	s C	vs	Ser	Ala	a Ar	or 175	er z	en	Len	212	71.0		ı Ala		٠ ـ ١	ַבַּב	ggt	532
79	5						9 +. ^	y	1311	rea	ALA			1 Ala	a Ph	ie I	Phe	Gly	
	•					8	U					85	;					90	
tto	: tt	c a	tt 9	gtg	tat	gc	a tt	ac	gt	gtg	aat	ctg	agt	gtt	90	a t	ta	ata	580
Phe	Ph	e I	le v	Val	Ty	Al.	a Le	eu A	rg	Val	Asn	Leu	Ser	· Val	ומ	- T	0	3-3 V-1	200
					95	5					100							val	
																1	05		
gat	ato	a at	a	ta t	tca														
Acr	Mo	- 1/2	.1 7	\	Con		. a.	a a		. La	gaa	gat	aat	aga	ac	t t	CC	aag	628
Joh	Me	L Ve	11 /	tsp	ser	ASI	ı Th	r T	hr 1	Leu	Glu	Asp	Asn	Arg	Th:	r s	er :	Lys	
			1	10					:	115					12	Ο.			
gcg	tgt	: cc	a g	rag	cat	tct	gc	t c	cc a	ata	aaa	gtt	cat	cat	aat	e c	aa :	200	676
Ala	Cys	Pr	0 G	lu	His	Ser	Al	a Pı	ro 1	lle	Lvs	Val	His	His	Agr		·	rb	070
		12	:5					1.3							- ASI	. G.	-11 1	ınr	
														135					
aat	220		a +	20	~~~					_									
Gly	7	7			caa	-	ga	g	ag	aa a	act	caa	gga	tgg	att	ct	c g	īgt	724
GLY	Lys	ь	SI	yr '	GIN	Trp	Asj	P Al	a G	lu :	Thr	Gln	Gly	Trp	Ile	: Le	eu G	ly	
	140	1			•		145	5					150					_	
tcc	ttt	tt	t t	at g	ggc	tac	ato	at	c a	ca d	ag a	att	cct	gga	aa.	ta	- -		770
Ser	Phe	Ph	e T	yr (ily	Tvr	Ile	11	е т	hr c	23 m	T 1 0-	Dro	Gly	234				772
155				•		160							PIO	GIY	GIY	17	r v	al	
						100					•	165					1	70	
7700	300																		
33-	agu	-	4 at	ag	133	999	aaa	at	g c	tg c	ta g	gga	ttt	9 99	atc	ct	t g	gc	820
Ala	Ser	Lys	s I,	le G	ly	Gly	Lys	Me	t L	eu L	eu (ly :	Phe	Gly	Ile	Le	u G	lv	
				1	.75						80			-		18			
	•																-		
act	gct	gto	ct	c a	cc	cta	ttc	act		- -	tt a	rct 4	703	gat					
Thr	Ala	Va 1	مرآ	11 T	hr	יים. זים.	Dha	Th-		-	2	1 -	gea '	yat -	cta -	99	a gt	-	868
			19				5 11¢	1111			те А	ma 1	ALA .	Asp :	Leu	Gl	y Va	al	
			13						19	₹5				:	200				
gga	cca	Ctc	at	t g	ta (ctc	aga	gca	ct	a g	aa g	ga d	ta 🤄	gga g	gag	ggt	: gt	t	916
Gly	Pro	Leu	Il	e V	al 1	Leu .	Arg	Ala	Le	u G	lu G	ly I	eu (Glv (Glu	Glv	, Va	1	
		205						210			_	•	:	, `	-	1		-	

aca Thr	Phe 220	Pro	gco Ala	a Mei	g car	gco s Ala 229	Met	tgg Tr	g to	t to:	t tgg Trp 230	Ala	cco Pro	c cc o Pr	t ctt o Leu	964
gaa Glu 235	aga Arg	ago Ser	aaa Lys	t Ctt	Let 240	ı Ser	: att	tcg Ser	tat Tyr	gca Ala 245	Gly	gca Ala	cag Glr	j cti Lěi	250	1012
aca Thr	gta Val	att Ile	ser	: ctt : Leu 255	Pro	ctt Leu	tct Ser	gga Gly	Ile 260	Ile	tgc Cys	tac Tyr	tat Tyr	Met 265	aat Asn	1060
				Phe											ttt Phe	1108
Leu	Leu	Trp 285	Ile	Trp	Leu	Val	Ser 290	Asp	Thr	Pro	Gln	Lys 295	His	Lys	aga Arg	1156
	Ser 300	His	Tyr	Glu	Lys	Glu 305	Tyr	Ile	Leu	Ser	Ser 310	Leu	Arg	Asn	Gln	1204
Leu 315	Ser	Ser	Gln	Lys	Ser 320	gtg Val	Pro	Trp	Val	Pro 325	Ile	Leu	Lys	Ser	Leu 330	1252
Pro	Leu	Trp	Ala	Ile 335	Val	gtt Val	Ala	His	Phe 340	Ser	Tyr	Asn	Trp	Thr 345	Phe	1300
Tyr	Thr	Leu	Leu 350	Thr	Leu	ttg Leu	Pro	Thr	Tyr	Met	Lys	Glu	Ile 360	Leu	Arg	1348
Phe .	Asn	Val 365	Gln	Glu	Asn		Phe : 370	Leu	Ser	Ser	Leu	Pro 375	Tyr	Leu	Gly	1396
	Trp 380	Leu	Суз	Met	Ile	Leu : 385	Ser (Gly (Gln .	Ala i	Ala /	Asp .	Asn .	Leu	Arg	1444
gca a Ala 1 395				Phe					Val i					Ser		1492

ata	gga	atç	g att	gga	3 CC1	gca	a gta	a tt	c ctg	gt	a gct	gct	gg:	c ttc	att	1540
Ile	Gly	/ Met	: Ile	e Gly	/ Pro	Ala	a Val	l Ph	e Lei	ı Va	l Ala	a Ala	GI	y Phe	T10	1340
				419					420					425		
	_								120	,				425		
ggc	tgt	gat	tat	CCT	ttg	gcc	gtt	gcı	tto	cta	a act	ata	tca	a aca	aca	1588
Gly	Cys	Asp	Tyr	Ser	Lei	ı Ala	. Val	. Ala	a Phe	Lei	ı Thr	Ile	Sei	r Thr	Thr	
			430)				439	5				440)		
ctq	qqa	qqc	ttt	tac	: tct	tct	ασа	LEER	ago	ato		cat	C=0	gat		
Len	GIV	์ เลา	Phe	Cve	Sar	Cor	- G1v	Dbo	Com	71.		· cac	٠٠٠	Asp	att	1636
	4 -7	445			001	501			: Jel	116	: ASII		Leu	ı Asp	Ile	
		443					450	+				455				
gct	cct	tcg	tat	gct	ggt	atc	ctc	ctg	ggc	ato	aca	aat	aca	ttt	gcc	1684
Ala	Pro	Ser	Tyr	Ala	Gly	Ile	Leu	Leu	Gly	Ile	Thr	Asn	Thr	Phe	Ala	
	460					465					470					
act	att	cca	gga	ato	att	aaa	ccc	ata	2++					acc		
The	Tla	D=0	994	Man	900	222	5	9.0	-1-	900	aaa	agt	ctg	acc	cct	1732
	116	PIO	GIY			GIA	Pro	vaı	тте		Lys	Ser	Leu	Thr	Pro	
475					480					485					490	
gat	aac	act	gtt	gga	gaa	tgg	caa	acc	gtg	ttc	tat	att	gct	gct	qct	1780
														Ala		
				495	· t	•			500		-4-			505		
														303		
att	225	at t	+++	~~+			++-									
T1 -	3	y	27.	990	900	-1	-1	-:	aca	cta -	LLC	gcc	aaa	ggt	gaa	1828
116	ASI	vai		GIY	Ala	TTE	Pne		Thr	Leu	Phe	Ala	Lys	Gly	Glu	
			510					515					520			
gta	caa	aac	tgg	gct	ctc	aat	gat	cac	cat	gga	cac	aga	cac			1870
									His							
		525	-				530			2		535		-		
												<i></i>				
tass	~~~															
cyaa	9944	icc a	dLdd	acaa	L CC	Lyce	LCLA	tta	atgt	att	ttta	ttta	tc a	tgta	acctc	1930
aaag	tgcc	tt c	tgta	ttgt	g ta	agca	ttct	atg	tctt	ttt	ttaa	ttgt	ac t	tgta	taga	1990
tttt	taag	gc c	tata	atca	t ga	aata	tcac	tag	ttgc	cag	aata	ataaa	aa t	gaact	atat	2050
									_	_				•	-3-3-	
ttaa	ttat	σа а	taat	atot	a ac	ctag	gact	tct	actti	- 20	atta					2110
		3		9-					4000	-49	gece	acaca	10 0	rgeet	geta	2110
a cca	ggca	ac a	cgaa	grag	gac	agtt	ctgt	tga	EEEE	ta	gggc	catac	t a	aaggg	aatg	2170
aget	gaaa	ca g	acct	cctg	a ta	cctti	tgct	taa	ttaaa	act a	agate	gataa	it t	ctcag	gtac	2230
														_	-	_
tgata	aaac	ac c	tatte	atta	t te	actt	cct	cata	aaaa	itt d	atcar	icter	C +	ctgac	20++	2200
			J:	٠ ي		,				;	a;	,		ccyac	actt	22 3 U

agaceteaaa etttageate tetgtggage tgecatecae tgtataattt egeetggeaa 2350

<210> 8

<211> 536

<212> PRT

<213> Homo sapiens

<400> 8

Met Ala Ala Gly Ala Met Thr Pro Pro Arg Pro Val Gln Pro Ala Arg

1 5 10 15

Pro Gly Gly Phe Gly Leu Ser Gly Arg Arg Ser Leu Leu Cys Gln Val

Ala Ser Thr Pro Ala His Val Gly Val Met Arg Ser Pro Val Arg Asp 35 40 45

Leu Ala Arg Asn Asp Gly Glu Glu Ser Thr Asp Arg Thr Pro Leu Leu 50 55 60

Pro Gly Ala Pro Arg Ala Glu Ala Ala Pro Val Cys Cys Ser Ala Arg 65 70 75 80

Tyr Asn Leu Ala Ile Leu Ala Phe Phe Gly Phe Phe Ile Val Tyr Ala 85 90 95

Leu Arg Val Asn Leu Ser Val Ala Leu Val Asp Met Val Asp Ser Asn 100 105 110

Thr Thr Leu Glu Asp Asn Arg Thr Ser Lys Ala Cys Pro Glu His Ser 115 120 125

- Ala Pro Ile Lys Val i.is His Asn Gln Thr Gly Lys Lys Tyr Gln Trp 130 135 140
- Asp Ala Glu Thr Gln Gly Trp Ile Leu Gly Ser Phe Phe Tyr Gly Tyr
 145 150 155 160
- Ile Ile Thr Gln Ile Pro Gly Gly Tyr Val Ala Ser Lys Ile Gly Gly
 165 170 175
- Lys Met Leu Gly Phe Gly Ile Leu Gly Thr Ala Val Leu Thr Leu 180 185 190
- Phe Thr Pro Ile Ala Ala Asp Leu Gly Val Gly Pro Leu Ile Val Leu 195 200 205
- Arg Ala Leu Glu Gly Leu Gly Glu Gly Val Thr Phe Pro Ala Met His 210 215 220
- Ala Met Trp Ser Ser Trp Ala Pro Pro Leu Glu Arg Ser Lys Leu Leu 225 230 235 240
- Ser Ile Ser Tyr Ala Gly Ala Gln Leu Gly Thr Val Ile Ser Leu Pro 245 250 255
- Leu Ser Gly Ile Ile Cys Tyr Tyr Met Asn Trp Thr Tyr Val Phe Tyr 260 265 270
- Phe Phe Gly Thr Ile Gly Ile Phe Trp Phe Leu Leu Trp Ile Trp Leu 275 280 285
- Val Ser Asp Thr Pro Gln Lys His Lys Arg Ile Ser His Tyr Glu Lys 290 295 300
- Glu Tyr Ile Leu Ser Ser Leu Arg Asn Gln Leu Ser Ser Gln Lys Ser 305 310 315 320
- Val Pro Trp Val Pro Ile Leu Lys Ser Leu Pro Leu Trp Ala Ile Val 325 330 335
- Val Ala His Phe Ser Tyr Asn Trp Thr Phe Tyr Thr Leu Leu Thr Leu 340 345 350
- Leu Pro Thr Tyr Met Lys Glu Ile Leu Arg Phe Asn Val Gln Glu Asn 355 360 365

Gly Phe Leu Ser Ser Leu Pro Tyr Leu Gly Ser Trp Leu Cys Met Ile 370 375 380

Leu Ser Gly Gln Ala Ala Asp Asn Leu Arg Ala Lys Trp Asn Phe Ser 385 390 395 400

Thr Leu Cys Val Arg Arg Ile Phe Ser Leu Ile Gly Met Ile Gly Pro
405 410 415

Ala Val Phe Leu Val Ala Ala Gly Phe Ile Gly Cys Asp Tyr Ser Leu 420 425 430

Ala Val Ala Phe Leu Thr Ile Ser Thr Thr Leu Gly Gly Phe Cys Ser 435 440 445

Ser Gly Phe Ser Ile Asn His Leu Asp Ile Ala Pro Ser Tyr Ala Gly
450 455 460

Ile Leu Leu Gly Ile Thr Asn Thr Phe Ala Thr Ile Pro Gly Met Val 465 470 475 480

Gly Pro Val Ile Ala Lys Ser Leu Thr Pro Asp Asn Thr Val Gly Glu 485 490 495

Trp Gln Thr Val Phe Tyr Ile Ala Ala Ile Asn Val Phe Gly Ala 500 505 510

Ile Phe Phe Thr Leu Phe Ala Lys Gly Glu Val Gln Asn Trp Ala Leu 515 520 525

Asn Asp His His Gly His Arg His 530 535

enter a complete de la complete de

<210> 9

<211> 1485

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: human/sheep consensus sequence

<220>

<221> CDS

<222> (1)..(1485)

< 40	0 > 9)														
atq	arq	tcy	, cc	gtt	ysı	gac	vt:	gco	csc	arc	: gav	, aac	. crac	י מפר	grgc	4.8
															Xaa	•
1				9		•			10			,				
-	-													15	,	
wca	ga c	cac	acı	- cck	c cts	ctr	· cma	sac	. acc						gct	0.0
															Ala	96
Add	vsħ	, vra	20		. AGC	. Add	, Add	25		, Ada	Arg	лаа			ALA	
			20	,				20	,				30)		
cca	~+~	+00	tac			cat	F 2.0		\/ + =						ttt	
															Phe	14
PIO	лаа	35		361	A10	ALG	40		Aaa	AIA	vaq			Pne	Pne	
		25					40					45				
		++0	~++	ete	+=+	kas	++2	aak	ata		ctg					
											Leu					19
GIY		FIIC	Add	Add		55	Deu	лаа	Val	Maii		лаа	vaı	хаа	xaa	
	50					23					60					
ata	an.	ato	atr	mat	tca	227	202	act	kam	~22	gat			1-		
											Asp					24
65	Add	MEC	AGG	AS P	70	naa	1111	1111	Add	75	-Asp	ASII	Arg	лаа	•	
0,5					,,					,,					80	
was	ama	tat	sca	gag	cat	tct	gct	ccc	ata	222	gtt	cwt	Cav	- 221/	633	200
											Val		_	-		28
		-1-		85					90	_,,	***	Add	naa	95	GIII	
									,,					,,,		
acσ	aat	aar	ааσ	tac	crr	taa	gat	gca	gaa	act	caa	gga	taa	att	ctc	336
											Gln					JJ.
			100	•		-	•	105					110			
ggw	tcy	ttt	tty	tat	ggc	tac	atc	atc	aca	car	att	cct	gga	σσa	tat	384
											Ile					
		115		-	-	•	120					125	2	3	-,-	
qtt	gcc	agc.	ara	akw	999	999	aar	mtg	yta	cta	gga	ttv	aaa	atc	vtt	432
											Gly					9 747 7
	130					135					140		3			
gsy	acw	gct	rtc	ytc	acc	ctg	ttc	act	ccc	mty	gct	qca	gat	ttm	gga	480
											Ala					
145					150					155			•		160	
gty	gga	scm	cty	rtt	gya	ctc	agr	gca	cta	gaa	ggr	cta	gga	gag	gat	528
											Xaa					
	•			165					170	· - -			3	175	1	
				_										•		
gty	aca	twt	cca	gcc	atg	cat	gcc	atq	tga	tct	tcw	tga	gct	ccc	cct	576
								_			Xaa		-			•
			180					185					190	· - -		

200	- ga	a ag	a ag	c aa	r ct	c ct	k ag	y at	t to	r ta	at go	ca go	ga qo	a ca	ar ct	t 62
Let	ı Gl	u Ar	g Se	r Xa	a Le	u Xa	a Xa	a Il	e Xa	a Ty	r A	la Gi	V A1	a Ya	aa Le	. 02
		19					20			•		20			-u DC	u
				•				•				~ `	, ,			
aac	a ac	a at	a rr	t tc	t ct	t cc										
233	, The	- Va	1 va	2 66	- 1-				- 99	a rt	a at	t tg	c ta	c ta	t at	672
GI			ı xa	a se	r Le			u Se	r Gi	у Ха	a Il	e Cy	s Ty	r Ty	r Mei	:
	210	0				21	5				22	0				
aat	: tg	gac	t ta	t gt	c tt	c ta	y tt	y tt	t gg	у ау	t rt	t gg	a at	m wt	y tgg	720
Asr	Tr	Th:	r Ty	r Va	l Phe	≥ Xaa	a Xa	a Phe	e Xa	a Xa	a Xa	a Gl	у Ха	а Ха	a Trp)
225					230					23			-		240	
																,
ttt	mtt	: tt:	t tac	ato	e tas	s tta	act	agt	יבה י						c aag	
Phe	Xaa	Xaa	Trr	Tle	Ya:	LOI	. Val	Co	· Ya	Th	- 1-	a 3a	a a	w ca	c aag s Lys	768
				245		· we	ı va.	. 361			L PI	O Aa	a Aa		_	1
				243	•				250	,				25	5	
															a aat	
Xaa	Xaa	Xaa	ı Xaa	Tyr	Glu	Lys	Xaa	Xaa	Ile	Lei	ı Se	r Sei	r Let	ı Xa	a Asn	
			260)				265					270)		
cag	cty	tct	tca	cag	aag	tca	gtg	ccg	tgg	rta	CC	atk	ytı	aaa	a tem	864
Gln	Xaa	Ser	Ser	Gln	Lys	Ser	Val	Pro	Trp	Xaa	ı Xaa	A Xaa	Xaa	LVS	3 Xaa	
		275			- 1		280		•			285		. , .	, Ada	
cta	cca	ctt	taa	act	atv	atm	att	aca	Cav		++				act	
) acc	912
	290	204	110	A.L.a	Add		Val	MIG	Add	Pne			ASI	Tr	Thr	
	230					295					300)				
			ttr													960
	171	Thr	Xaa	Leu	Xaa	Leu	Leu	Pro	Thr	Xaa	Met	Lys	Xaa	Xaa	Leu	
305					310					315					320	
agg	ttc	aat	rtt	caa	gag	aat	ggg	ttt	tta	tct	kca	kts	cct	tat	tta	1008
			Xaa													
				325					330					335		
Tav	tst	taa	tta	tat	ata	atc	cta	tck	aat	Caa	act		~~~			
(aa	Xaa	LAZ.	Leu	Cvo	Mor	Tla	Lau	Yaa	~i~	C1-	31-	33-	yac	aat	tta	1056
			340	cys	Mec	***	Deu		Gry	GIII	Ald	ALA	_	Asn	Leu	
			340					345					350			
					-											
•99	gca	ara	tgg	aat	ttt	tca	act	ytr	tgk	gtt	cgm	aga	rtt	ttt	agc	1104
lrg	Ala	Xaa	Trp	Asn	Phe	Ser	Thr	Xaa	Xaa	Val	Xaa	Arg	Xaa	Phe	Ser	
		355					360					365				
:tt	ata	ggr	atg	att	gga	cct	gcr	rta	ttc	ctq	gtw	qcv	acm	aam	ttv	1152
eu	Ile	Xaa	Met	Ile	Gly	Pro	Xaa	Xaa	Phe	Leu	Xaa	Xaa	Xaa	Xaa	Yas	-4-7-2
	370		_	_		375	_				380		-sua	DDA	aad	

atw	ggc	tgt	gat	tat	tcy	ttg	gcy	gtt	gcw	ttc	cta	acy	ata	tca	aca	1200
Xaa	Gly	Cys	Asp	Tyr	Xaa	Leu	Xaa	Val	Xaa	Phe	Leu	Xaa	Ile	Ser	Thr	
385					390					395					400	
	-															
acm	ctg	gġa	ggc	ttt	tgc	tct	tct	gga	ttt	agc	atc	aac	cat	ctq	gay	1248
Xaa	Leu	Gly	Gly	Phe	Cys	Ser	Ser	Gly	Phe	Ser	Ile	Asn	His	Leu	Xaa	
				405					410					415		
att	gct	cct	tcg	tat	gct	ggt	aty	ctc	ctq	ggc	atc	aca	aat	acm	ttt	1296
Ile	Ala	Pro	Ser	Tyr	Ala	Gly	Xaa	Leu	Leu	Glv	Ile	Thr	Asn	Xaa	Phe	2270
			420	_		-		425		2			430			
gcc	act	att	ccw	gga	atg	rtt	999	ccc	rtc	att	gcv	ara	agt	ctk	acc	1344
												Xaa				7344
		435		-										AGG	****	
		435					440					445				
		435					440					445				
cct	gak		act	rtt	gga	qaa		caa	acv	gtk	ttc		atv	act	act	1300
		aac					tgg					try				1392
		aac					tgg				Phe					1392
	Xaa	aac				Glu	tgg					try				1392
Pro	Xaa 450	aac Asn	Thr	Xaa	Gly	Glu 455	tgg Trp	Gln	Xaa	Xaa	Phe 460	try Xaa	Xaa	Ala	Ala	
Pro gct	Xaa 450 aty	aac Asn	Thr	Xaa ttt	Gly ggt	Glu 455 gcc	tgg Trp	Gln ttc	Xaa tty	Xaa aca	Phe 460 cta	try Xaa	Xaa gcc	Ala aaa	Ala	1392
Pro gct	Xaa 450 aty	aac Asn	Thr	Xaa ttt	Gly ggt	Glu 455 gcc	tgg Trp	Gln ttc	Xaa tty	Xaa aca Thr	Phe 460 cta	try Xaa	Xaa gcc	Ala aaa	Ala ggt Gly	
Pro gct Ala	Xaa 450 aty	aac Asn	Thr	Xaa ttt	Gly ggt Gly	Glu 455 gcc	tgg Trp	Gln ttc	Xaa tty	Xaa aca	Phe 460 cta	try Xaa	Xaa gcc	Ala aaa	Ala	
gct Ala 465	Xaa 450 aty Xaa	aac Asn aat Asn	Thr gtw Xaa	Xaa ttt Phe	ggt Gly 470	Glu 455 gcc Ala	tgg Trp att Ile	Gln ttc Phe	Xaa tty Xaa	Xaa aca Thr 475	Phe 460 cta Leu	try Xaa ttc Phe	Xaa gcc Ala	Ala aaa Lys	Ala ggt Gly	1440
gct Ala 465	Xaa 450 aty Xaa gtr	aac Asn aat Asn	Thr gtw Xaa	Xaa ttt Phe tgg	ggt Gly 470 gcy	Glu 455 gcc Ala mtc	tgg Trp att Ile	Gln ttc Phe	Xaa tty Xaa cac	Xaa aca Thr 475 caw	Phe 460 cta Leu	try Xaa ttc Phe	Xaa gcc Ala aga	Ala aaa Lys mac	Ala ggt Gly	
gct Ala 465	Xaa 450 aty Xaa gtr	aac Asn aat Asn	Thr gtw Xaa aac Asn	Xaa ttt Phe tgg	ggt Gly 470 gcy	Glu 455 gcc Ala mtc	tgg Trp att Ile	Gln ttc Phe gat Asp	Xaa tty Xaa cac His	Xaa aca Thr 475 caw	Phe 460 cta Leu	try Xaa ttc Phe	gcc Ala aga Arg	Ala aaa Lys mac Xaa	Ala ggt Gly	1440
gct Ala 465	Xaa 450 aty Xaa gtr	aac Asn aat Asn	Thr gtw Xaa aac Asn	Xaa ttt Phe tgg Trp	ggt Gly 470 gcy	Glu 455 gcc Ala mtc	tgg Trp att Ile	Gln ttc Phe gat Asp	Xaa tty Xaa cac	Xaa aca Thr 475 caw	Phe 460 cta Leu	try Xaa ttc Phe	gcc Ala aga Arg	Ala aaa Lys mac	Ala ggt Gly	1440

<210> 10

<211> 495

<212> PRT

<213> Artificial Sequence

<400> 10

Met Xaa Xaa Pro Val Xaa Asp Xaa Ala Xaa Xaa Xaa Gly Glu Glu Xaa 1 5 10 15

Xaa Asp Arg Xaa Xaa Xaa Xaa Xaa Ala Xaa Arg Xaa Glu Xaa Ala 20 25 30

Pro Xaa Cys Cys Ser Ala Arg Tyr Asn Xaa Ala Xaa Leu Xaa Phe Phe 35 40 45

Gly Phe Phe Xaa Xaa Tyr Xaa Leu Xaa Val Asn Leu Xaa Val Xaa Xaa 50 55 60

Val Xaa Met Xaa Asp Ser Xaa Thr Thr Xaa Xaa Asp Asn Arg Xaa Ser Xaa Xaa Cys Xaa Glu His Ser Ala Pro Ile Lys Val Xaa Xaa Xaa Gln Thr Gly Xaa Lys Tyr Xaa Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu Xaa Xaa Phe Xaa Tyr Gly Tyr Ile Ile Thr Xaa Ile Pro Gly Gly Tyr Val Ala Ser Xaa Xaa Gly Gly Xaa Xaa Xaa Leu Gly Xaa Gly Ile Xaa Xaa Xaa Ala Xaa Xaa Thr Leu Phe Thr Pro Xaa Ala Ala Asp Xaa Gly Xaa Gly Xaa Xaa Xaa Leu Xaa Ala Leu Glu Xaa Leu Gly Glu Gly Xaa Thr Xaa Pro Ala Met His Ala Met Trp Ser Xaa Trp Ala Pro Pro Leu Glu Arg Ser Xaa Leu Xaa Xaa Ile Xaa Tyr Ala Gly Ala Xaa Leu Gly Thr Val Xaa Ser Leu Pro Leu Ser Gly Xaa Ile Cys Tyr Tyr Met Asn Trp Thr Tyr Val Phe Xaa Xaa Phe Xaa Xaa Xaa Gly Xaa Xaa Trp Phe Xaa Xaa Trp Ile Xaa Leu Val Ser Xaa Thr Pro Xaa Xaa His Lys 245 ---- 255 ----Xaa Xaa Xaa Xaa Tyr Glu Lys Xaa Xaa Ile Leu Ser Ser Leu Xaa Asn Gln Xaa Ser Ser Gln Lys Ser Val Pro Trp Xaa Xaa Xaa Xaa Lys Xaa

Leu Pro Leu Trp Ala Xaa Xaa Val Ala Xaa Phe Ser Tyr Asn Trp Thr

Phe Tyr Thr Xaa Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xaa Leu

Arg Phe Asn Xaa Gln Glu Asn Gly Phe Leu Ser Xaa Xaa Pro Tyr Leu 325 330 335

Xaa Xaa Trp Leu Cys Met Ile Leu Xaa Gly Gln Ala Ala Asp Asn Leu
340 345 350

Arg Ala Xaa Trp Asn Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser 355 360 365

Leu Ile Xaa Met Ile Gly Pro Xaa Xaa Phe Leu Xaa Xaa Xaa Xaa Xaa 370 380

Xaa Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Xaa 405 410 415

Ile Ala Pro Ser Tyr Ala Gly Xaa Leu Leu Gly Ile Thr Asn Xaa Phe 420 425 430

Ala Thr Ile Xaa Gly Met Xaa Gly Pro Xaa Ile Xaa Xaa Ser Xaa Thr 435 440 445

Pro Xaa Asn Thr Xaa Gly Glu Trp Gln Xaa Xaa Phe Xaa Xaa Ala Ala 450 455 460

Ala Xaa Asn Xaa Phe Gly Ala Ile Phe Xaa Thr Leu Phe Ala Lys Gly 465 470 475 480

Glu Xaa Gln Asn Trp Xaa Xaa Xaa Asp His Xaa Gly His Arg Xaa 485 490 495

<210> 11

<211> 1485

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: human/sheep consencus sequence

<220>

<221> CDS

<222> (1)..(1485)

<400> 11

															g ng c	
Met	Xaa	a Xaa	a Pro	o Va	l Xa	a As	p Xa	a Al	a Xa	a Xa	a Xa	a Gly	/ Gl	u Gl	u Xaa	ì
1					5				1	0				1	5	
															c gct	
Xaa	Asp	Arg			a Xa	a Xa	a Xa			a Xa	a Arg	y Xaa	Gl	u Xa	a Ala	
			20	0				2	5				3	0		
															ttt	
Pro	хаа			s se	r Ala	a Arg			ı Xaa	a Ala	a Xaa			a Pho	e Phe	
		35	•				4()				45				
aat	F F C	++0	. ntt	ntr												
															nta Xaa	192
Gry	50		. Ado	. AGC	LIYI	. nae 55		l Add	vai	. ASI			val	. xaa	ı Xaa	
	50					33	,				60					
ata	gan	ato	atn	gat	. tca	aan	Laca	Lact	מחח	naa	gat	aat	202	. 201	tee	240
															Ser	240
65				•	70					75			9	nuo	80	
											•				00	
nan	gng	tgt	nca	gag	cat	tct	gct	ccc	ata	aaa	gtt	cnt	can	aan	caa	288
															Gln	
				85					90	-				95		
							•									
												gga				336
Thr	Gly	Xaa	Lys	Tyr	Xaa	Trp	Asp	Ala	Glu	Thr	Gln	Gly	Trp	Ile	Leu	
			100					105					110			
																•
												cct				384
xaa	хаа		Xaa	Tyr	GIY	Tyr		Ile	Thr	Xaa	Ile	Pro	Gly	Gly	Tyr	
		115					120					125				
a++	acc.	200	252			~~~										
												ttn Xaa				432
· 41	130	Ser		Aud	GIY	135	Add	Add	Add	reu		Aaa	GIA	TTE	хаа	
											140					
qnn	acn	qct	ntc	ntc	acc	cta	ttc	act	ccc	ntn	act	gca	gat	ttn	6 62	480
												Ala				400
145					150				-,	155	-				160	
			·												-00	
gtn	gga	ncn	ctn	ntt	gna	ctc	agn	gca	cta	gaa	ggn	cta	gga	gaq	ggt	528
												Leu				
				165					170				-	175	•	
												tgg (576
Kaa	Thr	Xaa	Pro	Ala	Met	His	Ala	Met	Trp	Ser	Xaa	Trp 2	Ala	Pro	Pro	
			100					100								

シ

ctt	gaa	a ag	a ag	c aa	n ct	t ct	n ag	n at	t to	n ta	it go	a gq	a go	a ca	an ct	t 624
Leu	Gli	ı Ar	g Se	r Xa	a Le	u Xa	a Xa	a Il	е ха	a Tv	r Al	a Gl	V Al	a Ya	aa Le	11
		19					20			•		20		- w Ac	ia De	u
								•				20	5			
aaa		. at	a nr:	t ta			<u>.</u>									
233	. The	. 17-	2 11C				- 25	ב נכי	t gg	a nt	a at	t tg	c ta	ic ta	t at	g 672
GIY			ı xa	a se:	r Le			u Se	r Gl	у Ха	a Il	е су	s Ty	T Ty	r Me	t
	210)				21	5				22	0				
aat	tgg	act	t tat	t gt	e tto	c ta	n tti	n tti	t gg:	n an	t nt	t gq	a at	n nt	n tg	g 720
Asn	Trp	Th	т Туз	r Val	l Phe	e Xa	a Xaa	a Phe	e Xaa	a Xa	а Ха	a Gl	v Xa	a Xa	a Tr	,
225					230					23			,		_	
					_										240	J
	ntt	++-	t e				. ~									
Dho	V			71-	. cgr		. 9	- agi	. yaı	ı ac	a CC	a na	a an	n ca	c aag	768
PILE	Add	Add	ıırı			ı Lei	ı val	. ser			r Pro	o Xaa	a Xa	a Hi	s Lys	3
				245	•				250)				25	5	
ana	atn	ncr	cnn	tat	gaa	aag	gar	tan	att	ctt	tça	a tca	a tt	a an	a aat	816
Xaa	Xaa	Xaa	Xaa	Tyr	Glu	Lys	Xaa	Xaa	Ile	. Let	ı Sei	r Sei	Le	u Xa	a Asr	ı
			260					265					27			-
caq	ctn	tct	tca	cag	aac	tca	ata	cca	taa	nta	CCT	a er			a ten	064
															x Cen	
	,,,,,,	275		0211	Lys	Jer			115	Aaa	. Add			а гуз	s Xaa	Į.
		2/3			· f		280					285	i			
	-															
															act	
Leu		Leu	Trp	Ala	Xaa	Xaa	Val	Ala	Xaa	Phe	Ser	Tyr	Asr	Tr	Thr	
	290					295					300)				
ttt	tat	act	ttn	ttg	acn	tta	ttg	cct	act	tan	atg	aag	gan	nto	cta	960
															Leu	
305					310					315		-4			320	
															320	
agg	ttc	aat	ntt	caa	σаσ	aat	aaa	+++	tta	tot	D.C.3				tta	
																1008
AL 9	FIIG	7011	-AGG		.G.Lu	ABII	GIY.	PILE		ser	Aaa	Xaa	ĽĽĢ	_	Leu	
				325					330					335	•	
															tta	1056
Xaa	Xaa	Trp	Leu	Cys	Met	Ile	Leu	Xaa	Gly	Gln	Ala	Ala	Asp	Asn	Leu	
			340					345					350			
							•									
agg	gca	ana	tgg	aat	ttt	tca	act	ntn	tgn	gtt	can	aga	ntt	ttt	age	1104
			Trp													
- J		355	F				360						nad	-WG	SEL.	
		- 					J J J					365				
			atg													1152
		xaa	Met	11e			Xaa	Xaa	Phe	Leu	Xaa	Xaa	Xaa	Xaa	Xaa	
	370					375					380					

atn	ggc	tgt	gat	tat	tcr	ı ttg	gcn	gtt	ger	tto	, cta	acn	ata	a tca	aca	1200
Xaa	Gly	Cys	Asp	Tyr	Xaa	Leu	Xaa	Val	. Xaa	Phe	Let	ı Xaa	Ile	Set	Thr	1100
385		•			390					395						
											•				400	
acn	GE C	gga	aac		+~-											
¥22	Tou	611			. cgc		-	gga	בכנ	ago	ato	aac	cat	ctg	gan	1248
лаа	Leu	GIY	GIY			Ser	Ser	Gly	Phe	Ser	Ile	Asn	His	Leu	Xaa	
				405					410					415		
att	gct	cct	tcg	tat	gct	ggt	atn	ctc	ctg	ggc	ato	aca	aat	acn	ttt	1296
Ile	Ala	Pro	Ser	Tyr	Ala	Gly	Xaa	Leu	Leu	Gly	Ile	Thr	Asn	Xaa	Phe	
			420					425		•			430			
					•								-50			
qcc	act	att	ccn	дда	atσ	ntt	aaa		ntc	a++	con	ana			_	
Ala	Thr	Tle	Yaa	Glv	Met	Yaa	222	חבים	Y	710	y	ana	age	cen.	acc	1344
		435	Aud	Gry	MEC	Add		Pro	xaa	TIE	хаа	Xaa		Xaa	Thr	
		433					440					445				
cct	gan	aac	act	ntt	gga	gaa	tgg	caa	acn	gtn	ttc	tnn	atn	gct	gct	1392
Pro	Xaa	Asn	Thr	Xaa	Gly	Glu	Trp	Gln	Xaa	Xaa	Phe	Xaa	Xaa	Ala	Ala	
	450					455					460					
gct	atn	aat	gtn	ttt	ggt	gcc	att	ttc	ttn	aca	cta	ttc	acc	222	aat	1440
Ala	Xaa	Asn	Xaa	Phe	Glv	Ala	Ile	Phe	Xaa	Thr	Len	Phe	λla	Luc	23.	1440
465					470					475		- 110	ALG	nys	-	
					•					4/3					480	
ga a	atn	C2 2	226	t.c.c												
												cac				1485
GIU	Add	GIN	ASN		xaa	xaa	xaa			Xaa	Gly	His	Arg	Xaa		
				485					490					495		

<210> 12

1

<211> 495

<212> PRT

<213> Artificial Sequence

<400> 12

Met Xaa Xaa Pro Val Xaa Asp Xaa Ala Xaa Xaa Xaa Gly Glu Glu Xaa 1 5 10 15

Xaa Asp Arg Xaa Xaa Xaa Xaa Xaa Ala Xaa Arg Xaa Glu Xaa Ala 20 25 30

Pro Xaa Cys Cys Ser Ala Arg Tyr Asn Xaa Ala Xaa Leu Xaa Phe Phe 35 . 40 . 45

Gly Phe Phe Xaa Xaa Tyr Xaa Leu Xaa Val Asn Leu Xaa Val Xaa Xaa 50 55 60

Val Xaa Met Xaa Asp Ser Xaa Thr Thr Xaa Xaa Asp Asn Arg Xaa Ser

6	5
---	---

1

70

75

80

Xaa Xaa Cys Xaa Glu His Ser Ala Pro Ile Lys Val Xaa Xaa Xaa Gln 85 90 95

Thr Gly Xaa Lys Tyr Xaa Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu 100 105 110

Xaa Xaa Phe Xaa Tyr Gly Tyr Ile Ile Thr Xaa Ile Pro Gly Gly Tyr 115 120 125

Val Ala Ser Xaa Xaa Gly Gly Xaa Xaa Xaa Leu Gly Xaa Gly Ile Xaa 130 135 140

Xaa Gly Xaa Xaa Xaa Leu Xaa Ala Leu Glu Xaa Leu Gly Glu Gly
165 170 175

Xaa Thr Xaa Pro Ala Met His Ala Met Trp Ser Xaa Trp Ala Pro Pro 180 185 190

Leu Glu Arg Ser Xaa Leu Xaa Xaa Ile Xaa Tyr Ala Gly Ala Xaa Leu 195 200 205

Gly Thr Val Xaa Ser Leu Pro Leu Ser Gly Xaa Ile Cys Tyr Tyr Met 210 215 220

Asn Trp Thr Tyr Val Phe Xaa Xaa Phe Xaa Xaa Xaa Gly Xaa Xaa Trp 225 230 235 240

Phe Xaa Xaa Trp Ile Xaa Leu Val Ser Xaa Thr Pro Xaa Xaa His Lys 245 250 255

Xaa Xaa Xaa Xaa Tyr Glu Lys Xaa Xaa Ile Leu Ser Ser Leu Xaa Asn 260 265 270

Gln Xaa Ser Ser Gln Lys Ser Val Pro Trp Xaa Xaa Xaa Xaa Lys Xaa 275 280 285

Leu Pro Leu Trp Ala Xaa Xaa Val Ala Xaa Phe Ser Tyr Asn Trp Thr 290 295 300

Phe Tyr Thr Xaa Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xaa Leu 305 310 315 320

Arg Phe Asn Xaa Gln Glu Asn Gly Phe Leu Ser Xaa Xaa Pro Tyr Leu

3	2	5
•	•	-

330

- Xaa Xaa Trp Leu Cys Met Ile Leu Xaa Gly Gln Ala Ala Asp Asn Leu 340 350
- Arg Ala Xaa Trp Asn Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser 355 360 365
- Leu Ile Xaa Met Ile Gly Pro Xaa Xaa Phe Leu Xaa Xaa Xaa Xaa Xaa 370 375 380
- Xaa Gly Cys Asp Tyr Xaa Leu Xaa Val Xaa Phe Leu Xaa Ile Ser Thr 385 390 395 400
- Xaa Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Xaa 405 410 415
- Ile Ala Pro Ser Tyr Ala Gly Xaa Leu Leu Gly Ile Thr Asn Xaa Phe 420 425 430
- Ala Thr Ile Xaa Gly Met Xaa Gly Pro Xaa Ile Xaa Xaa Ser Xaa Thr 435 440 445
- Pro Xaa Asn Thr Xaa Gly Glu Trp Gln Xaa Xaa Phe Xaa Xaa Ala Ala 450 455 460
- Ala Xaa Asn Xaa Phe Gly Ala Ile Phe Xaa Thr Leu Phe Ala Lys Gly 465 470 475 480
- Glu Xaa Gln Asn Trp Xaa Xaa Xaa Asp His Xaa Gly His Arg Xaa
 485 490 495

INTERNATIONAL SEARCH REPORT

International Application No

4 01 400			1/05 99/166/6
IPC 7	C12N15/12 C07K14/705 G01N3	3/50 A61K38/17	//C07K16/28
According	to International Patent Classification (IPC) or to both national class	ssification and IPC	
B. FIELDS	SEÄRCHED		
Minimum d IPC 7	ocumentation searched (classification system followed by classif C12N C07K G01N A61K	ication symbols)	
Documenta	ation searched other than minimum gocumentation to the extent to	hat such documents are included in	n the fields searched
Electronic o	data base consulted during the international search (name of dat	a base and, where practical, search	n terms used)
C. DOCUM	ENTS CONSIDERED TO BE RELEVANT		
Category '	Citation of document, with indication, where appropriate, of the	relevant passages	Relevant to claim No.
х	DATABASE EMBL - EMEST20 'Onlin Entry HS1173506, Acc.no. AA2585	ne! 513,	1,4-6
	19 March 1997 (1997-03-19) HILLIER, L. ET AL.: "zr59d01.r1 NhHMPu S1 Homo sapiens cDNA clo 5' similar to TR:G507415 G50741 SPECIFIC NA+-DEPENDENT INORGANI COTRANSPORTER." XP002121520 the whole document	one 667681 .5 BRAIN	
		-/	
_	· · · · · · · · · · · · · · · · · · ·		
X Furth	er documents are listed in the continuation of box C.	Patent family members	s are listed in annex.
"A" documer consider de filing da "L" documer which is citation "O" documer other m"P" documer	nt which may throw doubts on pnority claim(s) or s cited to establish the publication date of another or other special reason (as specified) nt referring to an oral disclosure, use, exhibition or	cited to understand the prin invention "X" document of particular relevication to econsidered nove involve an inventive step w "Y" document of particular relevicannot be considered to involve to ments ocument is combined with ments, such combination b in the art.	onflict with the application but incepted or theory underlying the ance; the claimed invention of or cannot be considered to then the document is taken alone ance; the claimed invention yolve an inventive step when the cone or more other such docueing obvious to a person skilled
	ctual completion of the international search	'&" document member of the sa	
5	November 1999	17/11/1999	auoriai search report
vame and m	alling address of the ISA European Patent Office. P B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (-31-70) 340-2040. Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Authorized officer Smalt, R	

L____

INTERNATIONAL SEARCH REPORT

International Application No

CICCON	DOCUMENTS CONCEDED TO	FUT/US 99	1/16676
Category	Citation of document, with indication where appropriate, of the relevant passages		I Salarana
	Specification of the contain pussages		Relevant to claim No.
Y	HELLEROVIST C G ET AL: "ANTITUMOR EFFECTS OF GBS TOXIN: A POLYSACCHARIDE EXOTOXIN FROM GROUP B BETA-HEMOLYTIC STREPTOCOCCUS" JOURNAL OF CANCER RESEARCH AND CLINICAL ONCOLOGY, vol. 120, no. 1/02. 1 January 1993 (1993-01-01), pages 63-70, XP000749401 ISSN: 0171-5216 the whole document		1-20,23, 25,26, 28-40
Y	GEARING, D.P. ET AL.: "Expression cloning of a receptor for human granulocyte-macrophage colony-stimulating factor." EMBO JOURNAL, vol. 8, no. 12, 1989, pages 3667-76, XP002121518 abstract		1-20,23, 25,26, 28-40
P,X	FU, C. ET AL.: "Expressional cloning of CM101 receptor gene from mammalian cells." PROCEEDINGS OF THE AMERICAN ASSOCIATION OF CANCER RESEARCH, vol. 40, March 1999 (1999-03), pages 557-Abstr.3677, XP002121519 the whole document		1-15
			: _V
			
. 8			
			*
- 1		1	

INTERNATIONAL SEARCH REPORT

iternational application No.

PCT/US 99/16676

Box I	Observations wher certain claims were found unsearchabl (Continuation 1 item 1 of first sheet)
This Inte	ernational Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X	Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely: see FURTHER INFORMATION SHEET PCT/ISA/210
2. X	Claims Nos.: because they relate to parts of the international Application that do not comply with the prescribed requirements to such an extent that no meaningful international Search can be carried out, specifically: Claims 24, 39, 40 and 44 could not be searched to completion due to insufficient characterization of the inhibitors in the description.
3.	Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box ii	Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This Inte	rnational Searching Authority found multiple inventions in this international application, as follows:
1.	As all required additional search fees were timely paid by the applicant, this international Search Report covers all searchable claims.
2	As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.	As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.	No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims: it is covered by claims Nos.:
Remark o	The additional search fees were accompanied by the applicant's protest. No protest accompanied the cayment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99 /16676

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 39, and 40 in as far as it pertains to an in vivo method, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Although claim 22, in as far as it relates to a method for use in vivo, is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Claims 24, 39, 40 and 44 could not be searched to completion due to insufficient characterization of the inhibitors in the description.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

-



WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau



	⁷ :	1 1	(11) International Publication Number: WO 00/05
C12N 15/12, C07K 14/705, C461K 38/17 // C07K 16/28	G01N 33/50,	A1	(43) International Publication Date: 3 February 2000 (03.0
21) International Application Number 22) International Filing Date:	r: PCT/US		BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI
30) Priority Data: 60/093,843 22 July 19 71) Applicant (for all designated St.	998 (22.07.98)	U VAN	MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, CY, DE
DERBILT UNIVERSITY [US Nashville, TN 37240 (US).			
(72) Inventors; and (75) Inventors/Applicants (for US only) [US/US]; 9014 Carondelet Pla (US). FU, Changlin [CN/US]; Brentwood, TN 37027 (US).	ce, Brentwood, T	'N 3702	With international search report.
(74) Agents: ASHTON, Nina, M.; Co El Camino Real, Five Palo Al 94306-2155 (US) et al.			
			·
54) Title: CRS TOYIN PECEPTOR			
(54) Title: GBS TOXIN RECEPTOR			
A novel GBS toxin receptor, and polypeptides are provided as well as of			tion and use are provided. GBS toxin receptor polynucleotide therapeutic methods and pharmaceutical compositions involving
57) Abstract A novel GBS toxin receptor, an olypeptides are provided as well as of			
A novel GBS toxin receptor, anolypeptides are provided as well as of			
57) Abstract A novel GBS toxin receptor, an olypeptides are provided as well as of	detection, screening		therapeutic methods and pharmaceutical compositions involving
A novel GBS toxin receptor, an olypeptides are provided as well as of	detection, screening	ng, and	therapeutic methods and pharmaceutical compositions involving
57) Abstract A novel GBS toxin receptor, an olypeptides are provided as well as of	detection, screening	ng, and	therapeutic methods and pharmaceutical compositions involving
57) Abstract A novel GBS toxin receptor, an olypeptides are provided as well as of	detection, screening	ng, and	therapeutic methods and pharmaceutical compositions involving
A novel GBS toxin receptor, and olypeptides are provided as well as colynucleotides and polypeptides.	detection, screening	ng, and	therapeutic methods and pharmaceutical compositions involving
A novel GBS toxin receptor, an olypeptides are provided as well as colynucleotides and polypeptides.	detection, screening	ng, and	therapeutic methods and pharmaceutical compositions involving
A novel GBS toxin receptor, and polypeptides are provided as well as coolynucleotides and polypeptides.	detection, screening	ng, and	therapeutic methods and pharmaceutical compositions involving
A novel GBS toxin receptor, and polypeptides are provided as well as coolynucleotides and polypeptides.	detection, screening	ng, and	therapeutic methods and pharmaceutical compositions involving

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	(L	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of Americ
CA	Canada	ΙT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG -	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		•
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

GBS TOXIN RECEPTOR

INTRODUCTION

Technical Field

5

10

15

20

25

This invention provides compositions and methods relating to GBS toxin receptor polynucleotides and polypeptides. The invention relates to a receptor for a polysaccharide isolated from a bacterial source.

Background

Group B \(\beta\)-hemolytic Streptococci (GBS) are ubiquitous microorganisms.

GBS is not known to cause any harmful infections in humans except for very young babies. GBS pneumonia, also called "early-onset disease", is associated with high morbidity and mortality in newborn infants.

In a series of studies conducted by Dr. Carl G. Hellerqvist and his associates at the Vanderbilt University School of Medicine, Nashville, Tennessee, a polysaccharide GBS toxin was identified. This toxin was determined to be a major factor in the complications of GBS pneumonia, and was found to be useful as a therapeutic agent in combating tumors though inhibition of vascularization (U.S. Patent No. 5,010,062).

In addition, as described in U.S. Patent No. 5,858,991 and WO98/32453, GBS toxin facilitates wound healing in patients by minimizing scarring and accelerating healing, and reduces wound-related tumor progression.

WO98/32452 and WO98/32448 describe the use of GBS toxin as a therapeutic agent for treating patients with chronic inflammatory diseases, such as rheumatoid arthritis and psoriasis, and for enhancing repair of neural injury.

Prior to this invention, receptors for GBS toxin had not been identified. The inventors, believing receptors of GBS toxin to reside on cells in the developing vasculature of tissues undergoing angiogenesis in the conditions described above, embarked upon a series of experiments resulting in the present invention.

SUMMARY OF THE INVENTION

For the first time, novel receptors for group B β-hemolytic Streptococcus GBS toxin (GBS toxin receptor) have been identified. One aspect of the invention provides a polypeptide comprising a GBS toxin receptor or polypeptide fragment thereof.

5 Preferred embodiments include mammalian GBS toxin receptors. Also provided is an antibody that recognizes GBS toxin receptor or a fragment thereof. The polypeptide of the invention can be used, *inter alia*, for the screening of compounds that can be used to treat or prevent conditions arising from pathologic or hypoxia-driven angiogenesis or neovascularization, such as, for example, cancerous tumors, chronic inflammatory disease, scarring during wound healing, keloids, neural injury, and reperfusion injury.

Another aspect of the invention provides a polynucleotide encoding a GBS toxin receptor or a fragment thereof and a polynucleotide hybridizable to such polynucleotide. Preferred polynucleotides are at least 10 bases in length and comprise a nucleic acid sequence encoding, or are complementary to a nucleic acid sequence encoding, a mammalian GBS toxin receptor or a polypeptide fragment thereof.

A third aspect of the invention is a complex comprising a GBS toxin bound to a mammalian toxin receptor or fragment thereof. Also provided is a method of forming such complex. The method comprises contacting a GBS toxin with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that permit specific binding of the GBS toxin to the polypeptide, and allowing the complex to form.

Yet another aspect of the invention is a method for purifying a compound that binds a GBS toxin receptor. The method comprises providing a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that binds GBS toxin, contacting the polypeptide with a sample comprising the compound under conditions that allow specific binding of the compound to the polypeptide, and separating the bound compound from the remainder of the sample.

Another aspect of the invention is a method of determining the presence or absence of GBS toxin in a sample. The method comprises contacting the sample with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that binds GBS toxin, under conditions that allow specific binding of GBS toxin to the GBS toxin receptor, and determining whether specific binding of GBS toxin has

15

20

25

occurred. Presence of GBS toxin in a sample obtained from a neonate is indicative of early onset disease.

A sixth aspect of the invention is a method for detecting pathologic vasculature in a mammalian tissue. The method comprises detecting the presence of a GBS toxin receptor. The method can be used for detecting or monitoring a variety of medical conditions associated with angiogenesis or neovascularization, such as, for example, detecting metastasis of a cancerous tumor, or monitoring the margin of a tumor in a mammal undergoing a therapy for cancer.

Another aspect of the invention provides methods for the identification of drug candidates for the treatment of medical conditions characterized by pathologic and/or hypoxia-driven angiogenesis or neovascularization. One embodiment is a method for identifying a compound that specifically binds a mammalian GBS toxin receptor. The method comprises combining a test compound with a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that allow specific binding to occur, and detecting a complex formed between the test compound and the polypeptide. Another embodiment is a method for determining cytotoxicity of a test chimeric compound. The method comprises exposing a cell expressing a mammalian GBS toxin receptor, or fragment thereof that binds GBS toxin, to a test chimeric compound comprising a cytotoxic agent coupled to GBS toxin, and detecting signs of toxicity. Yet another embodiment is a method for identifying an inhibitor of a GBS. toxin receptor by incubating test cells that express GBS toxin receptor, or a fragment thereof, in the presence and absence of a test compound and under conditions in which the cells incubated in the absence of the test compound can proliferate or migrate, and comparing the proliferation or migration of the test cells incubated in the presence and absence of the test compound, wherein less proliferation or migration in the presence of the test compound is indicative of the test compound being an inhibitor of the GBS toxin receptor. An inhibitor of endothelial cell proliferation or migration can be identified by the above method, wherein less proliferation or migration of test cells in the presence of the test compound is indicative of the test compound being an inhibitor of endothelial cell proliferation or migration. A therapeutic compound for the treatment or prevention of a medical condition characterized by pathologic angiogenesis or neovascularization can also be identified by the above method, wherein less proliferation or migration of test cells in the presence of the test compound is indicative of the test compound being a candidate

5

10

15

20

25

therapeutic compound for the treatment or prevention of the medical condition.

The invention also provides a method for identifying a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor. The method comprises simulating and selecting the most probable conformations of a mammalian GBS toxin receptor, designing a chemically modified analog that substantially mimics the energetically most probable three-dimensional structure of the polypeptide, chemically synthesizing the analog, and evaluating the bioactivity of the analog. Also provided is a method for identifying a compound which binds to a mammalian GBS toxin receptor. The method comprises simulating and selecting the most probable conformations of a mammalian GBS toxin receptor, deducing the most probable binding domains of the polypeptide, designing a compound that would form the energetically most probable complexes with the polypeptide, chemically synthesizing the compound, and evaluating the bioactivity of the compound.

Another aspect of the invention is a method for the prevention or treatment of neonatal onset disease in a human neonate by administering an inhibitor of binding of GBS toxin to a human GBS toxin receptor.

Yet another aspect of the invention is a method for inhibiting pathologic or hypoxia-driven endothelial cell proliferation or migration in a mammalian tissue. The method comprises specifically binding a molecule to a GBS toxin receptor present on the surface of at least one cell in the tissue, the molecule being selected from the group consisting of a compound that can evoke an inflammatory response when bound to a GBS toxin receptor in a mammal, a chimeric compound comprising a cytotoxic compound coupled to a compound that specifically binds the GBS toxin receptor, an inhibitor of GBS toxin receptor phosphorylation, and an inhibitor of GBS toxin receptor activity.

The invention also provides a GBS toxin receptor or fragment thereof, an inhibitor of a GBS toxin receptor, or an inhibitor of binding of a GBS toxin to a GBS toxin receptor, for use in a method of treatment of the human or animal body or for the manufacture of a medicament for the treatment of a medical condition characterized by pathologic or hypoxia-driven angiogenesis or neovascularization. Also provided is a chimeric compound comprising a cytotoxic agent coupled to a compound that binds GBS toxin receptor for use in a method of treatment of the human or animal body.

Also provided are pharmaceutical compositions comprising an inhibitor of a

5

10

15

20

25

GBS toxin receptor and/or a chimeric compound comprising a cytotoxic agent coupled to a compound that binds GBS toxin receptor, and a pharmaceutically acceptable carrier.

The invention also provides kits comprising a GBS toxin receptor or fragment
 and/or reagents for detecting the presence of a GBS toxin receptor or polypeptide
 fragment thereof or the presence of a polynucleotide encoding same.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts a process of rational drug design.

FIGS. 2A and 2B depict the results of immunohistochemical analysis of GBS toxin receptor expression in cancerous and normal human ovary tissue, respectively, using antibody Pab55 as described in Example 4.

FIGS. 3A and 3B depict the results of immunohistochemical analysis of GBS toxin receptor expression in cancerous and normal human ovary tissue, respectively, using antibody Pab57 as described in Example 4.

FIGS. 4A-4C depict the targeted delivery of a chimeric compound to GBS toxin receptor expressed in a cancerous tissue as described in Example 6.

DESCRIPTION OF SPECIFIC EMBODIMENTS

20 **DEFINITIONS**

10

Generally, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, and nucleic acid chemistry and hybridization described below are those well known and commonly employed in the art. Standard techniques are used for recombinant nucleic acid methods, polynucleotide synthesis, and microbial culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification steps supplied by manufacturers are typically performed according to the manufacturer's specifications. The techniques and procedures are generally performed according to conventional methods in the art and various general references (See generally, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring

25

Harbor, N.Y.) which are provided throughout this document. The nomenclature used herein and the laboratory procedures in analytical chemistry, organic synthetic chemistry, and pharmaceutical formulation described below are those well known and commonly employed in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical formulation and delivery, and treatment of patients. As employed throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

By "GBS toxin receptor" is meant a proteinaceous molecule capable of binding a toxin from Group B β-hemolytic *Streptococcus* bacteria (GBS toxin), such as, for example, CM101. A GBS toxin receptor is usually found in nature on the surface of a cell. Recombinant membrane bound and soluble GBS toxin receptors can be produced by laboratory techniques known in the art and described herein.

The term "isolated polynucleotide" referred to herein means a polynucleotide that has been subjected to manipulation, such that the isolated polynucleotide is no longer associated with the chromosome or cell that the polynucleotide is normally associated with in nature in the same manner as it is normally associated in nature. An example of an "isolated polynucleotide" is a polynucleotide of genomic, recombinant, or synthetic origin or some combination thereof.

The term "isolated protein" referred to herein means a protein that is no longer associated with the cell that the protein is normally associated with in nature in the same manner as it is normally associated in nature, such as (1) a protein free of at least some other proteins from the same source, (2) a protein expressed by a cell from a different species, (3) a protein that does not occur in nature, and (4) a protein produced from cDNA, recombinant RNA, or synthetic origin or some combination thereof.

The term "polypeptide" is used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein, fragments, and analogs are species of the polypeptide genus.

The term "naturally occurring" means found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) found in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

The term "operably linked" refers to a juxtaposition wherein the components

10

15

20

25

so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

The term "control sequence" refers to polynucleotide sequences which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is necessary for expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. The term includes single- and double-stranded forms of DNA.

The term "oligonucleotide" referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring and non-naturally occurring oligonucleotide linkages. An oligonucleotide is usually a polynucleotide 200 bases or fewer in length. Preferably oligonucleotides are minimally 10 to 60 bases in length and most preferably

15-35 bases in minimal length. Oligonucleotides are usually single-stranded, e.g. for probes; although oligonucleotides may be double-stranded, e.g. for use in the construction of a gene mutant. Oligonucleotides of the invention can be either sense or antisense oligonucleotides. The term "naturally occurring nucleotides" referred to herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoroaniladate, phosphoroamidate, and the like. An oligonucleotide can include a label for detection, if desired.

5

10

15

20

25

By "complementary" or "complement" is meant that wherever adenine appears in a first nucleic acid sequence, thymine or uracil is found in the "complementary" sequence and vice versa, and wherever guanine appears in a first nucleic acid sequence, cytosine is found in the "complementary" sequence and vice versa.

The term "sequence identity" describes the proportion of base matches between two nucleic acid sequences or the proportion of amino acid matches between two amino acid sequences, i.e. the degree of identity between two sequences. When sequence identity is expressed as a percentage, e.g., 50%, the percentage denotes the proportion of exact matches over the length of sequence from a GBS toxin receptor sequence that is compared to some other sequence. Various computer alignment programs can be used to determine sequence identity. In its simplest form, % identity is calculated by dividing the number of exact matches between two nucleic acid sequences or between two amino acid sequences by the total number of nucleotides or amino acids in the reference sequence. For example, if there are 300 matches between sequences 400 amino acids in length, the sequences have 75% identity. Uracil and thymine are considered identical when comparing a ribonucleic acid sequence with a deoxyribonucleic acid sequence.

As applied to polynucleotides, the term "substantial identity" means that two nucleic acid sequences when optimally aligned, such as by the program BLAST (Altschul et al., *J. Mol. Biol.* 215:403-410 (1990)), share at least about 85%, preferably at least about 90% sequence identity and most preferably 95% or greater sequence identity. When using computer alignment programs, gaps (in either of the two sequences) are permitted to maximize matching; gap lengths of 15 bases or less are usually used; 6 bases or less are preferred; 2 bases or less are most preferred. When using oligonucleotides as probes or in treatments, the sequence identity between the target nucleic acid and the oligonucleotide sequence is generally not less than 17 target base matches out of 20 possible oligonucleotide base pair matches (85%); preferably not less than 9 matches out of 10 possible base pair matches (90%), and most preferably not less than 19 matches out of 20 possible base pair matches (95%).

5

10

15

20

25

Preferably, bases which are not identical nevertheless are part of a degenerate codon that encodes the same amino acid at that amino acid position. Alternatively, bases which are not identical preferably are part of a degenerate codon that encodes a conservative amino acid substitution for that amino acid position.

As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned by the BLAST computer program, share at least about 80 percent sequence identity, preferably at least about 86 percent sequence identity, more preferably at least about 95 percent sequence identity, even more preferably at least about 99 percent sequence identity up to having one amino acid difference, and most preferably share 100% identity. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine. alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatichydroxyl side chains is serine and threonine; a group of amino acids having amidecontaining side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acid substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamic-aspartic, and asparagine-glutamine.

The term "hybridizable under high stringency conditions" referred to herein means capable of specific binding under conditions whereby only nucleic acid sequences having a substantial identity of greater than 95% with respect to each other will hybridize. These conditions are known in the art and discussed herein.

The term "degenerate codon" means any of the nucleotide codon triplets encoding a desired amino acid according to the genetic code. Codons can be selected based upon known preferred codon usage in a host organism such as *E. coli*.

The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-

5

10

15

20

- 25

occurring sequence deduced, for example, from a full-length DNA sequence. Fragments typically are at least 3 amino acids long, preferably are 5-10 amino acids long, more preferably are 10-50 amino acids long, even more preferably are more than 50 amino acids long and comprise at least one extracellular domain of a GBS toxin receptor. Most preferred are fragments that comprise the entire extracellular domains of a GBS toxin receptor, and preferably also comprise portions of transmembrane and intracellular domains sufficient to maintain the polypeptide fragment in a functional stereochemical conformation on the surface of a cell, lipid membrane, liposome, micelle, or other lipophilic structure.

The term "immunologically reactive" means having antigenic properties or being capable of being specifically bound by an antibody that can specifically bind GBS toxin receptor. A substance has antigenic properties if it can generate monoclonal or polyclonal antibodies when administered to an animal under conditions known in the art to facilitate the production of antibodies that will recognize and bind a particular antigen.

A "heterologous polypeptide" is a polypeptide different from polypeptides normally produced by a particular cell. For example, a GBS toxin receptor polypeptide or fragment thereof that is produced recombinantly in a cell that does not normally produce such GBS toxin receptor polypeptide or fragment thereof, is a heterologous polypeptide. A second polypeptide joined to a GBS toxin receptor polypeptide or fragment thereof is also a heterologous polypeptide if it is not joined to a GBS toxin receptor polypeptide in nature.

As used herein, the terms "label" or "labeled" refers to incorporation of a detectable marker, e.g., by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes (e.g., ³H, ¹⁴C, ³⁵S, ¹²⁵I, ¹³¹I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, \(\beta\)-galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some

5

10

15

20

25

embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

The term "compound" as used herein preferably refers to a peptidic, peptidomimetic, organic, or other chemical molecule and also refers to a nucleic acid molecule or chemical derivative thereof. The compound can interact with, or be, the polynucleotides or polypeptides of the invention.

The singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise.

The SEQ ID NOs of the nucleic acid and amino acid sequences described herein are summarized below in Table 1.

Table 1
Nucleic Acid and Amino Acid Sequences

SEQ ID NO:	Type of Sequence	Description
SEQ ID NO: 1	nucleic acid	Partial human GBS toxin receptor (HP55)
SEQ ID NO: 2	amino acid	Partial human GBS toxin receptor (HP55)
SEQ ID NO: 3	nucleic acid	Sheep GBS toxin receptor (SP55)
SEQ ID NO: 4	amino acid	Sheep GBS toxin receptor (SP55)
SEQ ID NO: 5	nucleic acid	Primer
SEQ ID NO: 6	nucleic acid	Primer
SEQ ID NO: 7	nucleic acid	Full-length human GBS toxin receptor (HP59)
SEQ ID NO: 8	amino acid	Full-length human GBS toxin receptor (HP59)
SEQ ID NO: 9	nucleic acid	Human/Sheep consensus GBS toxin receptor
		coding region
		(with base codes a, c, g, t, m, r, w, s, y, k)
SEQ ID NO: 10	amino acid	Human/Sheep consensus GBS toxin receptor
		coding region (translation of SEQ ID No: 9)
SEQ ID NO: 11	nucleic acid	Human/Sheep consensus GBS toxin receptor
		coding region
		(with base codes a, c, g, t, n)
SEQ ID NO: 12	amino acid	Human/sheep consensus GBS toxin receptor
		coding region (translation of SEQ ID NO: 11)

The headings provided herein describe the general topic discussed and are not intended to be exclusive of information discussed in other sections. Frequently, information, methods, compositions, and other aspects may be applicable to more than one embodiment of the invention and can be so combined.

Introduction

20 GBS toxin binds to tissues undergoing pathologic, hypoxia-driven, and

embryologic angiogenesis or neovascularization. The inventors have identified at least two mammalian GBS toxin receptors, which are described herein. Examples 1 and 2 describe the cloning and characterization of some GBS toxin receptors. The inventors have classified GBS toxin receptor as an integral protein with seven transmembrane domains. The predicted segments are shown in Table 7. The protein has several putative sites for phosphorylation by cAMP-dependent kinase, protein kinase C (PKC), and casein kinase II (CK2). Typically, such integral proteins, upon binding of a molecule (e.g., a ligand or an extracellular messenger), undergo a conformational change which facilitates phosphorylation at phosphorylation sites such as those discussed above. The phosphorylation of the protein at these sites may trigger a signal transduction cascade, which often results in proliferation or other nuclear responses of the cells which have been exposed to the binding molecule. Angiogenesis or neovascularization involves proliferation and migration of endothelial cells. As discussed in greater detail in Examples 4 and 5, GBS toxin receptor expression is correlated with medical conditions involving pathologic, hypoxia-driven, and embryogenic angiogenesis or neovascularization. GBS toxin receptor polypeptides can be used for a variety of purposes, including screening for compounds that can inhibit endothelial cell proliferation and/or migration mediated by GBS toxin receptor and screening for cytotoxic chimeric compounds that can bind to and destroy cells expressing GBS toxin receptor. GBS toxin receptor polynucleotides can be used for a variety of purposes, including the design of antisense polynucleotides that can block translation of messenger RNA encoding GBS toxin receptor.

25 POLYNUCLEOTIDES

One aspect of the invention provides for isolated polynucleotides at least ten bases in length encoding or complementary to a nucleic acid sequence encoding a GBS toxin receptor or a fragment derived therefrom. Preferably, the GBS toxin receptor is a mammalian GBS toxin receptor, more preferably an ovine, bovine or feline GBS toxin receptor, and most preferably a human GBS toxin receptor. The isolated polynucleotides can be naturally occurring or non-naturally occurring. The isolated polynucleotides can comprise a DNA sequence or an RNA sequence in which every T is replaced with U. For purposes of determining percentage identity, T is considered equivalent to U. Preferably, the polynucleotides include alleles of an

30

10

15

ovine, bovine, feline or human GBS toxin receptor, and can include alleles of GBS toxin receptor of other mammals. These polynucleotides can be isolated using polynucleotides derived from SEQ ID NOs: 1, 3, 7, 9 and 11, as described further below.

Polynucleotides, oligonucleotides and fragments of the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. The polynucleotides can be hybridizable under high stringency conditions to a nucleic acid molecule having a nucleic acid sequence comprising at least 20 contiguous polynucleotides, preferably at least 30 contiguous nucleotides of SEQ ID NO: 1 or SEO ID NO: 3, and even more prefereably to the nucleic acid sequence of SEO ID NO: 1, 3, 7, 9 or 11 or the complement of SEQ ID NO: 1, 3, 7, 9 or 11. Such polynucleotides can be used for performing selective, high stringency hybridization and are particularly useful for performing amplification of nucleic acid by polymerase chain reaction (PCR) to determine the presence or absence of GBS toxin receptor in a sample, for isolating a naturally occurring nucleic acid encoding a GBS toxin receptor (see Example 3), as antisense molecules for blocking translation of GBS toxin receptor mRNA. Particularly preferred are polynucleotides hybridizable under high stringency conditions to a nucleic acid molecule having a nucleic acid sequence comprising the nucleic acid sequence of nucleotides 266 to 1870 of SEQ ID NO: 7 (the putative full length coding region of a human GBS toxin receptor, excluding the start codon), nucleotides 266 to 1870 of SEQ ID NO:7 (the putative full length coding region of a human GBS toxin receptor, including the start codon), nucleotides 61 to 1542 of SEQ ID NO:1 (the partial coding region of a human GBS toxin receptor, excluding the start codon), nucleotides 58 to 1542 of SEQ ID NO: 1 (the partial coding region of a human GBS toxin receptor, including the start codon), nucleotides 87 to 1568 of SEQ ID NO: 3 (the coding region of a sheep GBS toxin receptor, excluding the start codon), nucleotides 84 to 1568 of SEQ ID NO:3 (the coding region of a sheep GBS toxin receptor, including the start codon), or a complementary nucleic acid sequence thereof.

The polynucleotides can have an identity to the nucleic acid sequence of a corresponding region of SEQ ID NO: 1, 3 or 7 or the complement of a corresponding region of SEQ ID NO: 1, 3 or 7 in the range of about 85% to 100%, preferably greater than about 87% identity, more preferably greater than about 95% identity, and most

5

10

15

20

25

preferably about 99% to 100% identity. Particularly preferred are polynucleotides comprising the nucleic acid sequence of nucleotides 266 to 1870 of SEQ ID NO: 7, or nucleotides 87 to 1568 of SEQ ID NO: 3, SEQ ID NO: 9, SEQ ID NO:11, or a complementary nucleic acid sequence thereof.

Preferably, the polynucleotides comprise a nucleic acid sequence encoding, or complementary to a nucleic acid sequence encoding, a polypeptide having an identity to the amino acid sequence of a fragment of a GBS toxin receptor in the range of about 85% to 100%, more preferably greater than 86% identity, even more preferably greater than 95% identity, and most preferably 99% to 100% identity. Preferably, the fragment binds GBS toxin. Preferred fragments comprise all or a portion of residues 1 to 495 of SEQ ID NO: 2 or all or a portion of residues 1 to 536 of SEQ ID NO: 8. Particularly preferred are polynucleotides comprising a nucleic acid sequence encoding a polypeptide having 100% identity to the amino acid sequence of residues 1 to 495 of SEQ ID NO: 4, residues 1 to 495 of SEQ ID NO: 2, or residues 1 to 536 of SEQ ID NO:8.

Polynucleotides encoding naturally occurring GBS toxin receptor can be isolated from various tissue sources and cell cultures from different species that produce such a receptor by the methods described herein, such as, for example, cells from tumor endothelium, synovial tissue in rheumatoid arthritis, or hypoxic tissue deprived of or restricted from blood flow, such as in reperfusion injury or wounded tissue. Such polynucleotides can be isolated by hybridization using probes or by polymerase chain reaction using oligonucleotides, as well as by implementing other molecular biology techniques known in the art. Such probes and oligonucleotides typically comprise various regions of the sequence of SEQ ID NO: 1, 3, 7, 9 or 11, preferably of SEQ ID NO: 1, 3, or 7, or encode various regions of the sequence of SEQ ID NO. 2, 4, 8,10 or 12, preferably of SEQ NO: 2, 4 or 8.

Polynucleotides useful for cloning genes encoding GBS toxin receptors of various organisms can be determined by comparing the amino acid sequences of homologous proteins. (see Table 4). For example, conserved regions can be targeted for the synthesis of oligonucleotides or degenerate oligonucleotides to be used as probes for hybridization or nucleic acid amplification, techniques discussed further below and in Example 3. Stringency can be varied to achieve selective hybridization conditions whereby nucleic acid sequences having less than 95% identity with respect to each other will hybridize. These conditions are known in the art and discussed

5

10

15

20

25

herein and examples are provided. Generally, the nucleic acid sequence identity between the polynucleotides, oligonucleotides, and fragments of the invention and a nucleic acid sequence of interest will be at least about 85%, and more typically with preferably increasing identities of at least about 90%, 95%, 99%, and 100%.

Polynucleotides can be used as probes under high stringency wash conditions and with corresponding hybridization conditions, as known in the art. Small polynucleotides, for example, polynucleotides 200 bases or fewer in length, are often referred to in the art as oligonucleotides. Techniques for using polynucleotides as probes to detect the same or related nucleic acid sequences is well known in the art. See, for example, Sambrook et al, especially Chapter 11, the text of which is herein incorporated by reference. Usually, probes can be made from polynucleotides that are 10 to 200 bases in length. Preferably probes are made from polynucleotides 10 to 60 nucleotides in length and most preferably 12 to 40 bases in length. Specific probes can be designed based on results obtained using nucleic acid homology computer programs such as FASTA, which uses the method of Pearson and Lipman (Proc. Natl. Acad. Sci. USA 85:2444-2448 (1988)) and shows the degree of identity between compared sequences. The size of the probe is dependent upon the region of the gene to which it will be hybridized. The size of the probe increases as the degree of homology to undesirable nucleic acid sequences increases. A probe 10-50 nucleotides in length can be used, preferably more than 50 nucleotides, even more preferably more than 100 nucleotides, and most preferably a probe made from the entire coding region of a GBS toxin receptor will be used. To decrease the number of false positives, preferably two probes are used to identify clones that bind to both probes under hybridization and wash conditions. Oligonucleotides can be synthesized on an Applied BioSystems oligonucleotide synthesizer according to specifications provided by the manufacturer.

Typically, hybridization and washing conditions are performed at according to conventional hybridization procedures. Typical hybridization conditions for screening plaque lifts (Benton and Davis (1978) *Science* 196: 180) can be: 50% formamide, 5 x SSC (sodium chloride, sodium citrate) or SSPE (sodium chloride, sodium phosphate, EDTA), 1-5 x Denhardt's solution, 0.1-1% SDS, 100-200 μg sheared heterologous DNA or tRNA, 0-10% dextran sulfate, 1 x 10⁵ to 1 x 10⁷ cpm/ml of denatured probe with a specific activity of about 1 x 10⁸ cpm/ μg , and

5

10

15

20

25

incubation at 42°C for about 6-36 hours. Prehybridization conditions are essentially identical except that probe is not included and incubation time is typically reduced. Washing conditions are typically 1-3 x SSC, 0.1-1% SDS, 42-70°C with change of wash solution at about 5-30 minutes. Cognate bacterial sequences, including allelic sequences, can be obtained in this manner. For high stringency hybridization conditions, various parameters can be altered to increase the stringency of hybridization, such as by increasing the temperature of incubation with the labeled probe. Preferably, for greater flexibility in experimental design, the probe can be hybridized at a lower temperature, such as, for example, room temperature and the stringency can then be modified by altering the salt concentration and temperature of the wash solutions. For high stringency a wash temperature of greater than or equal to 42°C can be used, such as, for example, 68°C, in a wash buffer having a salt concentration less than 3X SSC, such as, for example, 0.1X SSC. In some cases. TMACL can also be used, particularly for polynucleotides rich in G-C base pairs in order to decrease non-specific binding. A lower stringency wash can be used to hybridize polynucleotides with lower identities or polynucleotides that are less than 60 base pairs in length. For a low stringency wash, temperatures of less than or equal to 42° can be used in a wash buffer having a salt concentration of greater than or equal to 2X SSC.

The invention includes methods for amplification of target nucleic acids, such as the polymerase chain reaction ("PCR") technique. The PCR technique can be applied to identify related sequences in the genomes of various organisms and to detect nucleotide sequences in suspected samples, using oligonucleotide primers spaced apart from each other and based on the genetic sequence set forth herein. The primers are complementary to opposite strands of a double-stranded DNA molecule and are typically separated by from about 50 to 450 nucleotides or more (usually not more than 2000 nucleotides). This method entails preparing the specific oligonucleotide primers followed by repeated cycles of target DNA denaturation, primer binding, and extension with a DNA polymerase to obtain DNA fragments of the expected length based on the primer spacing. Extension products generated from one primer serve as additional target sequences for the other primer. The degree of amplification of a target sequence is controlled by the number of cycles that are performed and is theoretically calculated by the simple formula 2n where n is the number of cycles. Given that the average efficiency per cycle ranges from about 65%

5

10

15

20

25

to 85%, 25 cycles produce from 0.3 to 4.8 million copies of the target sequence. The PCR method is described in a number of publications, including Saiki et al., Science (1985) 230:1350-1354; Saiki et al., Nature (1986) 324:163-166; and Scharf et al., Science (1986) 233:1076-1078. Also see U.S. Patent Nos. 4,683,194; 4,683,195; and 4,683,202, the text of each patent is herein incorporated by reference. Additional methods for PCR amplification are described in: PCR Technology: Principles and Applications for DNA Amplification ed. HA Erlich, Freeman Press, New York, NY (1992); PCR Protocols: A Guide to Methods and Applications, eds. Innis, Gelfland, Snisky, and White, Academic Press, San Diego, CA (1990); Mattila et al. (1991)

Nucleic Acids Res. 19: 4967; Eckert, K.A. and Kunkel, T.A. (1991) PCR Methods and Applications 1: 17, and; PCR, eds. McPherson, Quirkes, and Taylor, IRL Press, Oxford, all of which are incorporated herein by reference.

In yet another embodiment, an antisense polynucleotide can be administered to a mammal to treat or prevent a medical condition involving pathologic and/or hypoxia-driven angiogenesis. The antisense oligonucleotides of the invention can be 15 synthesized by any of the known chemical oligonucleotide synthesis methods. Such methods are generally described, for example, in Winnacker, From Genes to Clones: Introduction to Gene Technology. VCH Verlagsgesellschaft mbH (H., Ibelgaufts trans. 1987). Any of the known methods of oligonucleotide synthesis can be utilized in preparing the instant antisense oligonucleotides. The antisense oligonucleotides are 20 most advantageously prepared by utilizing any of the commercially available, automated nucleic acid synthesizers. The device utilized to prepare the oligonucleotides described herein, the Applied Biosystems 380B DNA Synthesizer, utilizes -cyanoethyl phosphoramidite chemistry. Antisense oligonucleotides hybridizable with any portion of the mRNA transcript can be prepared by the 25 oligonucleotide synthesis methods known to those skilled in the art. While any length oligonucleotide can be utilized in the practice of the invention, sequences shorter than 12 bases may be less specific in hybridizing to the target GBS toxin receptor mRNA, and may be more easily destroyed by enzymatic digestion. Hence, oligonucleotides having 12 or more nucleotides are preferred. Sequences longer than 18 to 21 30 nucleotides may be somewhat less effective in inhibiting GBS toxin receptor translation because of decreased uptake by the target cell. Thus, oligomers of 12-21 nucleotides are most preferred in the practice of the present invention, particularly oligomers of 12-18 nucleotides. Oligonucleotides complementary to and hybridizable

with any portion of the GBS toxin receptor mRNA transcript are, in principle, effective for inhibiting translation of the transcript, and capable of inducing the effects herein described. Translation is most effectively inhibited by blocking the mRNA at a site at or near the initiation codon. Thus, oligonucleotides complementary to the 5' region of the GBS toxin receptor mRNA transcript are preferred. Secondary or tertiary structure which might interfere with hybridization is minimal in this region. Moreover, sequences that are too distant in the 3' direction from the initiation site can be less effective in hybridizing the mRNA transcripts because of a "read-through" phenomenon whereby the ribosome is postulated to unravel the antisense/sense duplex to permit translation of the message. (see, e.g. Shakin, J. Biochemistry 261, 16018 (1986)). The antisense oligonucleotide is preferably directed to a site at or near the ATG initiation codon for protein synthesis. Oligonucleotides complementary to a portion of the GBS toxin receptor mRNA including the initiation codon are preferred. While antisense oligomers complementary to the 5' region of the GBS toxin receptor transcript are preferred, particularly the region including the initiation codon, it should be appreciated that useful antisense oligomers are not limited to those complementary to the sequences found in the translated portion of the mRNA transcript, but also includes oligomers complementary to nucleotide sequences contained in, or extending into, the 5' and 3' untranslated regions. Antisense nucleotides or antisense expression constructs can find use to treat or prevent diseases associated with pathologic or hypoxia-driven angiogenesis and neovascularization, as inappropriate expression of GBS toxin receptor results in hyperproliferation of endothelial cells.

In one embodiment, the polynucleotides of the invention can exist in linear form. In another embodiment, the polynucleotides can exist in circular form as part of a plasmid.

In yet another embodiment, a probe or PCR primer comprises a group of polynucleotide species containing different degenerate codons at various positions, which polynucleotides encode, or are complementary to sequences encoding, a GBS toxin receptor in whole or in part. Such polynucleotides can be useful for isolating nucleic acid sequences encoding polypeptides having at least about 85% identity to the amino acid sequence of sheep or human GBS toxin receptor, such as, for example, GBS toxin receptors of other organisms. Typically, such polynucleotides are synthesized chemically as described above by programming a synthesizer to incorporate a particular combination of nucleic acid residues at a certain position.

5

10

15

20

25

Typical designations are shown in Table 2.

Table 2

Base Codes

Symbol	Meaning
Α	A: adenine
С	C; cytosine
G	G; guanine
T	T; thymine
U	U; uracil
M	A or C
R	A or G
W	A or T/U
S	C or G
Y	C or T/U
K	G or T/U
V	A or C or G; not T/U
Н	A or C or T/U; not G
D	A or G or T/U; not C
В	C or G or T/U; not A
N	A or C or G or T/U

POLYPEPTIDES

5

10

15

20

25

30

Another aspect of the invention provides polypeptides comprising (1) the full length GBS toxin receptor protein or a naturally occurring allelic variant thereof. (2) fragments of at least 3 amino acids of the amino acid sequence of SEQ ID NO: 2, 4, 8, 10 or 12, and (3) a GBS toxin receptor protein, polypeptide, or polypeptide fragment having an amino acid identity in the range of about 80% to 100% to the amino acid sequence of a corresponding region of SEQ ID NO: 2, 4 or 8. Preferred fragments of the amino acid sequence of SEQ ID NO: 2, 4, 8, 10 or 12, are at least 5, 6, 7, 8 or 9 amino acids in length and are immunologically reactive, i.e., immunogenic. More preferred are fragments at least 25 amino acids in length and fragments comprising the amino acid sequence of residues 181 to 419 of SEQ ID NO: 2 or residues 1 to 240 of SEQ ID NO: 4. Most preferred are fragments that can bind GBS toxin. Preferably, the GBS toxin receptor protein, polypeptide, or polypeptide fragment has an amino acid identity to the amino acid sequence of a corresponding region of SEO ID NO: 2. 4 or 8 of at least about 86%, more preferably at least about 95% identity, even more preferably at least about 99% identity up to having one amino acid difference, and most preferably 100% identity. Preferred polypeptides have at least about 89% identity, more preferably at least about 95% identity, even more preferably at least about 99% identity up to having one amino acid difference, and most preferably 100% identity to the amino acid sequence of residues 181 to 419 of SEQ ID NO: 2, residues 1 to 495 of SEQ ID NO: 4. Preferably, a full length GBS toxin receptor protein comprises the amino acid sequence of residues 1 to 495 of SEO ID NO: 2, residues 1 to 495 of SEQ ID NO: 4, or residues 1 to 536 of SEQ ID NO: 8, or an allelic variant thereof. The polypeptides of the invention can include amino acids in addition to the GBS toxin receptor protein, polypeptide, or polypeptide fragment. Such polypeptides typically comprise a heterologous polypeptide joined to a second polypeptide derived. as described above, from a GBS toxin receptor. Preferably the additional amino acids are covalently linked to the amino-terminal or carboxy-terminal terminus of the GBS toxin receptor protein, polypeptide, or polypeptide fragment.

Fragments or analogs of GBS toxin receptor can be prepared by those of ordinary skill in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. For example, such functional domains include domains conferring the property of induction of an inflammatory response upon binding of GBS toxin to the GBS toxin receptor. GBS toxin mediates

the binding and opsonization by C3 of endothelial cells that express the GBS toxin receptor. Such domains can comprise the binding site for GBS toxin, in whole or in part, or domains otherwise essential for GBS toxin receptor structure and/or function. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known (Bowie et al. (1991) *Science* 253: 164). Computerized prediction methods, such as, for example, a hydropathy profile as provided by the "Soap" program in PC/GENE can be employed to identify putative structural and functional domains. Using the method of Klein, Kanehisa and DeLise, Biochim Biophys Acta (1985) 815:468-476, the inventors have classified a sheep GBS toxin receptor, SP55, as an integral protein with seven transmembrane segments predicted. Such a protein is also known colloquially in the art as a "7-spanner". The predicted segments are set forth below in Table 3.

Table 3

Predicted Transmembrane Domains of SP55

	Inner Boundaries		Outer Boundaries		Segment	P:I odds*
No.	From	To	From	To	Sequence	
1	232	248	226	252	FFGIVGIIWFILWICLV (232-248 of SEQ ID No. 4)	2.589323E-05
2	369	385	365	389	LIGMIGPAIFLVAAGFI (369-385 of SEQ ID No. 4)	1.007311E-03
3	458	474	456	479	TVFCIAAAINVFGAIFF (458-474 of SEQ ID No. 4)	2.482542E-03
4	137	153	135	157	LLLGFGIFATAIFTLFT (137-153 of SEQ ID No. 4)	7.564906E-03
5	42	58	42	58	LAFLSFFGFFVLYSLRV (42-58 of SEQ ID No. 4)	8.236557E-02
6	328	344	328	345	GFLSAVPYLGCWLCMI L (328-344 of SEQ ID No. 4)	.1925022
7	390	406	390	407	SLAVAFLTISTTLGGFC (390-406 of SEQ ID No. 4)	.8064944

^{*} Relates hydrophobicity of integral sequence to the hydrophobicity of the peripheral sequence. An integral sequence with a higher hydrophobicity number is more likely to be part of a transmembrane domain.

A computerized alignment of the amino acid sequences of GBS toxin receptor in various organisms provides further guidance in preparing preferred fragments. See, for example, Table 4 which compares the amino acid sequence of residues 42 to 536

20

5

of a human GBS toxin receptor (HP59) (residues 42 to 536 of SEQ ID NO: 8) and a sheep GBS toxin receptor (SP55).

Table 4 Alignment of Human and Sheep GBS Toxin Receptor Amino Acid Sequences

5	Sequences	
SP55	MKSPVSDLAPSDGEEGSDRTPLLQRAPRAEPAPVCCSARYNLAFLSFFGF	50
HP55	MRSPVRDLARNDGEESTDRTPLLPGAPRAEAAPVCCSARYNLAILAFFGF	50
SP55	FVLYSLRVNLSVALVDMVDSNTTAKDNRTSYECAEHSAPIKVLHNQTGKK	100
HP55	FIVYALRVNLSVALVDMVDSNTTLEDNRTSKACPEHSAPIKVHHNQTGKK	100
SP55	YRWDAETQGWILGSFFYGYIITQIPGGYVASRSGGKLLLGFGIFATAIFT	150
HP55	YQWDAETQGWILGSFFYGYIITQIPGGYVASKIGGKMLLGFGILGTAVLT	150
SP55	LFTPLAADFGVGALVALRALEGLGEGVTYPAMHAMWSSWAPPLERSKLLS	200
HP55	LFTPIAADLGVGPLIVLRALEGLGEGVTFPAMHAMWSSWAPPLERSKLLS	200
SP55	ISYAGAQLGTVVSLPLSGV CYYMNWTYVFYFFG VG IWF LW CLVSD	250
HP55	ISYAGAQLGTVISLPLSGIICYYMNWTYVFYFFGTIGIFWFLLWIWLVSD	250
SP55	TPETHKTITPYEKEYILSSLKNQLSSQKSVPWIPMLKSLPLWAIVVAHFS	300
HP55	TPQKHKRISHYEKEYILSSLRNQLSSQKSVPWVPILKSLPLWAIVVAHFS	300
SP55	YNWTFYTLLTLLPTYMKEVLRFNIQENGFLSAVPYLGCWLCMILSGQAAD	350
HP55	YNWTFYTLLTLLPTYMKEILRFNVQENGFLSSLPYLGSWLCMILSGQAAD	350
SP55	NLRARWNFSTLWVRRVFSLIGMIGPAIFLVAAGFIGCDYSLAVAFLTIST	400
HP55	NLRAKWNFSTLCVRRIFSLIGMIGPAVFLVAAGFIGCDYSLAVAFLTIST	400
SP55	TLGGFCSSGFSINHLDIAPSYAGILLGITNTFATIPGMIGPIIARSLTPE	450
HP55	TLGGFCSSGFSINHLDIAPSYAGILLGITNTFATIPGMVGPVIAKSLTPD	450
SP55	NTIGEWQTVFCIAAAINVFGAIFFTLFAKGEVQNWAISDHQGHRN	495
H P5 5	NTVGEWQTVFYIAAAINVFGAIFFTLFAKGEVQNWALNDHHGHRH	495

HP55 - SEQ ID NO: 2 SP55 - SEQ ID NO: 4

Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in a GBS toxin receptor sequence.

Although one class of preferred embodiments are fragments having aminoand/or carboxy-termini corresponding to amino acid positions near functional domains borders, alternative fragments may be prepared. The choice of the aminoand carboxy-termini of such fragments rests with the discretion of the practitioner and will be made based on experimental considerations, such as ease of construction. stability to proteolysis, thermal stability, immunological reactivity, amino- or carboxyl-terminal residue modification, or other considerations. Polypeptide fragments usually contain at least nine amino acids and can contain any number of amino acids provided that the peptide fragment is at least about 80% identical to the corresponding fragment of SEO ID NO: 2, SEO ID NO: 4, or SEO ID NO:8. The human GBS toxin receptor has 41 additional amino acids on the N-terminus compared to the sheep GBS toxin receptor (compare SEQ ID NO:4 and SEQ ID NO:8). Analogs can comprise additions or deletions of some or all of those 41 N-terminal amino acids. N-terminal and C-terminal additions useful, e.g., for purification and/or antibody recognition are also contemplated. Examples include histidine tags, a FLAG (phenylalanine, leucine, alanine, guanine) epitope, fusion partners such as glutathione S transferase, chloramphenicol acetyltransferase (CAT), luciferase, β-galactosidase, and the like. Deletions of unconserved amino acids are also contemplated, provided that the structural integrity and/or binding properties of the GBS toxin receptor are not substantially compromised.

Analogs can also comprise amino acid substitutions, preferably conservative substitutions. Also preferred are conservative and/or non-conservative substitutions in regions having less shared identity among various species. For example, a variant of a GBS toxin receptor can comprise conservative and/or non-conservative substitutions of amino acids corresponding to residues 2, 6, 10, 11, 16, 17, 24, 31, 44, 46, 52, 53, 55, 74, 75, 81, 82, 84, 93, 102, 132, 133, 137, 144, 145, 148, 149, 155, 159, 163, 165, 166, 179, 212, 219, 235, 236, 239, 242, 246, 253, 254, 257, 259, 260, 271, 283, 285, 319, 324, 332, 333, 338, 355, 362, 366, 377, 439, 442, 445, 450, 453, 461, 487, 488, 491 and 495 of SEQ ID NO:4. Preferably the substitution is an amino acid present in the corresponding position of SEQ ID NO:4 or SEQ ID NO:8. For example, referring to the alignment plot in Table 4, the amino acid corresponding to position 152 of SEQ ID NO:4 can be arginine (R), glutamine (Q), or a conservative or non-conservative substitution of R or Q, and preferably is R or Q. Such regions can

5

10

15

be identified by amino acid sequence alignment plots, such as that shown in Table 4. Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for GBS toxin, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various mutations of a sequence other than the naturally-occurring peptide sequence, such as, for example, single or multiple amino acid substitutions.

A conservative amino acid substitution should generally not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, disrupt disulfide bonds or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles, (1984) Creighton (ed.), W.H. Freeman and Company, New York; Introduction to Protein Structure, (1991), C. Branden and J. Tooze, Garland Publishing, New York, NY; and Thornton et al. (1991) Nature 354: 105 (which are incorporated herein by reference). A conservative substitution is a "replacement of an amino acid in a polypeptide by one with similar characteristics." (McGraw-Hill Dictionary of Scientific and Technical Terms, Fifth Edition, 1994, Sybil P. Parker, Editor in Chief). The structure and characteristics of naturally occurring amino acids has long been known in the art (Biochemistry, Second Edition, Albert L. Lehninger, 1975, pages 71-76) For example, amino acids which are similar by virtue of their hydrophobic R groups are alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, and methionine. Alanine, valine, leucine, and isoleucine are similar by virtue of their aliphatic R groups. Phenylalanine and tryptophan are similar by virtue of their aromatic R groups. Glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine are similar by virtue of their uncharged polar R groups. Glycine and alanine are similar by virtue of their small size. Serine and threonine are similar by virtue of a hydroxyl in their R group. Asparagine and glutamine differ by only one methyl group. Similarly, aspartic acid and glutamic acid differ by only one methyl group, and they are similar by virtue of their acidic R groups. Lysine, arginine, and histidine are similar by virtue of their basic R groups. In addition, lysine and arginine are similar by virtue of the amino groups on the end of the aliphatic chain in their R groups. Tyrosine and phenylalanine are similar by virtue of their aromatic groups.

5

10

15

20

25

Amino substitutions commonly made in the art include a substitution of valine for leucine or isoleucine, alanine for glycine, serine for threonine, asparagine for glutamine, aspartic acid for glutamic acid, and lysine for arginine, tyrosine for phenylalanine, and vice versa.

Typically, one skilled in the art would generally refrain from changing amino acids that are conserved among the various GBS toxin receptors, but a conservative substitution might reasonably be made. For example, Table 4 guides one skilled in the art to avoid substitutions, particularly nonconservative substitutions, for amino acids corresponding to residues 1, 3-5, 7-9, 12-15, 18-23, 26-30, 32-43, 45, 47-51, 54, 56-73, 76-80, 83, 85-92, 94-101, 103-131, 134-136, 138-143, 146-147, 150-154, 156-158, 160-162, 164, 167-178, 180-211, 213-218, 220-234, 237-238, 240-241, 243-245, 247-252, 255-256, 258, 261-270, 272-282, 284, 286-318, 320-323, 325-331, 334-337, 339-354, 356-361, 363-365, 367-376, 378-438, 440-441, 443-444, 446-449, 451-452, 454-460, 462-486, 489-490 and 492-494 of SEQ ID NO:4, which are conserved among the GBS toxin receptors shown in Table 4.

Tables 5 and 6 describe sequences within HP59 and SP55, respectively, that match predicted amidation, N-glycosylation, cAMP-phosphorylation, CK2-phosphosylation, myristylation (addition of unsaturated fatty acid molecules), and PKC-phosphosylation sites (Omega 1.1 sequence analysis program). The information contained in these tables provides guidance to one skilled in the art for designing GBS toxin receptor variants and fragments. When designing polypeptide variants, for example, one may decide to avoid substitutions in some or all of these regions. When designing polypeptide fragments other than immunogenic polypeptide fragments, for example, one may opt to include some or all of these regions.

Tab Putative Reco in H	gaition Sites		Table Putative Recog in SP	gnition Sites	
Site	Seq. ID NO: 8 Residues:	Sequence	Site	Seq. ID NO: 4 Residues:	Sequence
AMIDATION	23-26	SGRR	AMIDATION	97-100	TGKK
AMIDATION	138-141	TGKK	ASN_GLYCOSYLATION	59-62	NLSV
ASN GLYCOSYLATION	100-103	NLSV	ASN_GLYCOSYLATION	71-74	NTTA
ASN GLYCOSYLATION	112-115	NTTL	ASN_GLYCOSYLATION	77-80	NRTS
ASN GLYCOSYLATION	118-121	NRTS	ASN_GLYCOSYLATION	95-98	NQTG
ASN_GLYCOSYLATION	136-139	NQTG	ASN_GLYCOSYLATION	225-228	NWTY
ASN GLYCOSYLATION	266-269	NWTY	ASN_GLYCOSYLATION	302-305	NWTF
ASN GLYCOSYLATION	343-346	NWTF	ASN_GLYCOSYLATION	357-360	NFST
ASN_GLYCOSYLATION	398-401	NFST	CK2_PHOSPHO_SITE	11-14	SDGE
CAMP_PHOSPHO_SITE	297-300	KRIS	CK2_PHOSPHO_SITE	73-76	TAKD
CK2_PHOSPHO_SITE	113-116	TTLE	CK2_PHOSPHO_SITE	79-82	TSYE

5

10

15

Putative Rec	ole 5 ognition Sites IP59		Table 6 Putative Recognition Sites in SP55			
	Seq. 1D NO: 8			Seq. ID NO: 4		
Site	Residues:	Sequence	Site	Residues:	Sequence	
CK2_PHOSPHO_SITE	114-117	TLED	CK2_PHOSPHO_SITE	259-262	TPYE	
CK2_PHOSPHO_SITE	300-303	SHYE	CK2_PHOSPHO_SITE	452-455	TIGE	
CK2_PHOSPHO_SITE	493-496	TVGE	MYRISTYL	126-131	GGYVAS	
MYRISTYL	66-71	GAPRAE	MYRISTYL	142-147	GIFATA	
MYRISTYL	167-172	GGYVAS	MYRISTYL	162-167	GALVAL	
MYRISTYL	183-188	GILGTA	MYRISTYL	172-177	GLGEGV	
MYRISTYL	213-218	GLGEGV	MYRISTYL	205-210	GAQLGT	
MYRISTYL	246-251	GAQLGT	MYRISTYL	209-214	GTVVSL	
MYRISTYL	250-255	GTVISL	MYRISTYL	337-342	GCWLCM	
MYRISTYL	378-383	GSWLCM	MYRISTYL	386-391	GCDYSL	
MYRISTYL	427-432	GCDYSL	MYRISTYL	403-408	GGFCSS	
MYRISTYL	444-449	GGFCSS	MYRISTYL	423-428	GILLGI	
MYRISTYL	46 4-46 9	GILLGI	MYRISTYL	427-432	GITNTF	
MYRISTYL	468-473	GITNTF	PKC_PHOSPHO_SITE	17-19	SDR	
PKC_PHOSPHO_SITE	23-25	SGR	PKC_PHOSPHO_SITE	37-39	SAR	
PKC_PHOSPHO_SITE	58 -6 0	TDR	PKC_PHOSPHO_SITE	55-57	SLR	
PKC_PHOSPHO_SITE	78-80	SAR	PKC_PHOSPHO_SITE	73-75	TAK	
PKC_PHOSPHO_SITE	120-122	TSK	PKC_PHOSPHO_SITE	97-99	TGK	
PKC_PHOSPHO_SITE	138-140	TGK	PKC_PHOSPHO_SITE	254-256	THK	
PKC_PHOSPHO_SITE	310-312	SLR	PKC_PHOSPHO_SITE	269-271	SLK	
PKC_PHOSPHO_SITE	317-320	SQK	PKC PHOSPHO SITE	276-278	SQK	

In light of the foregoing, preferred polypeptides comprise an amino acid sequence of the formula:

AA1-AAn-AAm

5 wherein:

10

15

20

25

AA1 is absent or is M;

AAn is a contiguous chain of 0 to 100 amino acids, preferably of 0 or 41 amino acids, even more preferably of residues 2-42 of SEQ ID NO:8; and

AAm is a contiguous chain of 494 amino acids comprising AA43 through AA536, wherein:

(1) each of AA43, AA47, AA51, AA52, AA57, AA58, AA65, AA66, AA72, AA85, AA87, AA93, AA94, AA96, AA115, AA116, AA122, AA123, AA125, AA134, AA143, AA173, AA174, AA178, AA185, AA186, AA189, AA190, AA196, AA200, AA204, AA206, AA207, AA220, AA253, AA260, AA276, AA277, AA280, AA283, AA287, AA294, AA295, AA298, AA300, AA301, AA312, AA324, AA326, AA360, AA365, AA373, AA374, AA379, AA396, AA403, AA407, AA418, AA480, AA483, AA486, AA491, AA494, AA502, AA528, AA529, AA532 and AA536 is an essential amino acid or a modified amino acid and preferably is an amino acid residue corresponding to:

(a) residue 43, 47, 51, 52, 57, 58, 65, 66, 72, 85, 87, 93, 94, 96, 115, 116, 122, 123, 125, 134, 143, 173, 174, 178, 185, 186, 189, 190, 196, 200, 204, 206, 207, 220, 253, 260, 276, 277, 280, 283, 287, 294, 295, 298, 300, 301, 312, 324, 326, 360, 365, 373, 374, 379, 396, 403, 407, 418, 480, 483, 486,

491, 494, 502, 528, 529, 532 and 536, respectively, of SEO ID NO:8: (b) residue 2, 6, 10, 11, 16, 17, 24, 25, 31, 44, 46, 52, 53, 55, 74, 75, 81, 82, 84, 93, 102, 132, 133, 137, 144, 145, 5 148, 149, 155, 159, 163, 165, 166, 179, 212, 219, 235, 236. 239, 242, 246, 253, 254, 257, 259, 260, 271, 283, 285, 319, 324, 332, 333, 338, 355, 362, 366, 377, 439, 442, 445, 450, 453, 461, 487, 488, 491 and 495, respectively of SEQ ID NO:4; or 10 (c) a conservative substitution thereof: (2) each of AA44-AA46, AA48-AA50, AA53-AA56, AA59-AA64, AA67-AA71, AA73-AA84, AA86, AA88-AA92, AA95, AA97-AA114, AA117-AA121, AA124, AA126-AA133, AA135-AA142, AA144-AA172, AA175-AA177, AA179-AA184, AA187-15 AA188, AA191-AA195, AA197-AA199, AA201-AA203, AA205. AA208-AA219, AA221-AA252, AA254-AA259, AA261-AA275, AA278-AA279, AA281-AA282, AA284-AA286, AA288-AA293. AA296-AA297, AA299, AA302-AA311, AA313-AA323, AA325, AA327-AA359, AA361-AA364, AA366-AA372, AA375-AA378, 20 AA380-AA395, AA397-AA402, AA404-AA406, AA408-AA417. AA419-AA478, AA481-AA482, AA484-AA485, AA487-AA490, AA492-AA493, AA495-AA501, AA503-AA527, AA530-AA531 and AA533-AA535 is (a) residue 44-46, 48-50, 53-56, 59-64, 67-71, 73-84. 25 86, 88-92, 95, 97-114, 117-121, 124, 126-133, 135-142, 144-172, 175-177, 179-184, 187-188, 191-195, 197-199, 201-203. 205, 208-219, 221-252, 254-259, 261-275, 278-279, 281-282, 284-286, 288-293, 296-297, 299, 302-311, 313-323, 325, 327-359, 361-364, 366-372, 375-378, 380-395, 397-402, 404-406, 30 408-417, 419-478, 481-482, 484-485, 487-490, 492-493, 495-501, 503-527, 530-531 and 533-535, respectively, of SEQ ID NO:8; or

(b) a conservative substitutions thereof; and (3) AA315 through AA367 are optionally absent.

Preferred polypeptides comprise the amino acid sequence of SEQ ID NO:4, SEQ ID NO:8 or an amino acid sequence which varies from that sequence only at the specific residues which are not conserved between the sheep GBS toxin receptor (SEQ ID NO:4) and the human GBS toxin receptor (SEQ ID NO:8). Of those variations, the most preferred variations are those resulting in a polypeptide encoded by SEQ ID NO:11. Even more preferred variations are those amino acids in the corresponding positions of the amino acid sequence of SEQ ID NO:4. Particularly preferred are polypeptides comprising an amino acid sequence that differs from SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:8 at no more than about 20% of the amino acid residues, with increasing preference for no more than about 10%, 5%, 1%, with one to zero amino acid differences being most preferred.

Besides targeting specific amino acids for change, analogs of GBS toxin receptor can also be prepared by techniques involving activity selection, such as, for example, phage display, directed evolution, DNA shuffling, and homologous in procaryotes or eucaryotes of genes from different species, as described in part in U.S. Patent Nos. 5,605,793; 5,830,721; 5,811,238; 5,837,458; 5.093,257; 5,223,409; 5,403,484; 5,571,698; and 5,837,500, which are incorporated herein by reference.

Any variant or fragment of the human and sheep GBS toxin receptors described herein can be tested for the requisite activity by determining whether the variant or fragment can bind GBS toxin.

These polypeptides provide reagents useful in drug discovery and purification and can be used in various *in vitro* assays, preferably when expressed on the surface of a cell, e.g., a stable transfected cell. For example, assays such as binding assays can be used to screen test compounds, including polysaccharides and other compounds, for their ability to bind the GBS toxin receptor. Assays can identify potential drug candidates that block GBS toxin binding to the GBS toxin receptor. Such drugs are useful for preventing and/or treating early onset disease in neonatal humans. Some polypeptides can be used to competitively inhibit binding GBS toxin to a GBS toxin receptor.

5

10

The polypeptides of the invention can be used to affinity purify GBS toxin, a GBS toxin chimeric compound, and other polysaccharides or compounds which can bind the GBS toxin receptor.

The polypeptides can also be used to develop a method of targeting a cytotoxic agent for delivery to a cell that expresses a GBS toxin receptor. For example, a cytotoxic agent can be coupled to a molecule that binds a GBS toxin receptor for selective delivery to the neovasculature of a growing tumor. Such a delivery system would permit a highly concentrated, localized attack on a growing tumor, while minimizing the adverse systemic side effects encountered with most chemotherapeutics. In one instance, the cytotoxic agent can be GBS toxin, which, upon binding to GBS toxin receptor, induces an inflammatory response as described in Hellerqvist et al., Angiogenesis: Molecular Biology, Clinical Aspects, Edited by M.E. Maragoudakis et al., Plenum Press, New York 1994, pp. 265-269. In a similar manner, selective delivery of a therapeutic agent to a cell that expresses a GBS toxin receptor could be used advantageously to treat tumors, rheumatoid arthritis or neural injury, or to facilitate wound healing.

The polypeptides of the invention can also be used to screen for and/or design a GBS toxin mimetic with improved therapeutic properties, such as, for example, improved ability to inhibit hypoxia-induced neovascularization or angiogenesis. Such mimetics are useful in the treatment and prevention of conditions resulting from hypoxia-induced neovascularization or angiogenesis, such as, for example, tumor growth, scarring during wound healing, gliosis during repair of neural injury, reperfusion injury, restenosis, rheumatoid arthritis, psoriasis, other chronic inflammatory diseases characterized by angiogenesis, etc. Therapeutic properties can be improved by enhancing biological stability, affinity for the GBS toxin receptor, complement binding activity, reducing antigenicity, etc.

The polypeptides of the invention can also be used to generate antibodies for various therapeutic and research purposes. The polypeptides of the invention can be used to immunize rabbits, mice, goats, chickens, or other animals known in the art to be amenable to such immunization. Monoclonal antibodies are generally preferred but polyclonal antibodies can also be used, provided that detection of binding of the GBS toxin receptor antibody to the GBS toxin receptor is possible. The production of non-human monoclonal antibodies, e.g., murine, is well known (see, e.g., Harlow et al., Antibodies A Laboratory Manual, Cold Spring Harbor Press, pp. 139-240, 1989,

5

10

15

20

25

incorporated herein by reference). As it may be difficult to generate human monoclonal antibodies to a human receptor or binding domain polypeptide, it may be desirable to transfer antigen binding regions of non-human monoclonal antibodies, e.g. the F(ab')₂ or hypervariable regions or murine monoclonal antibodies, to human constant regions (Fc) or framework regions by recombinant DNA techniques to produce substantially human molecules. Such methods are generally known and are described in, e.g., U.S. Pat. Nos. 4,816,397 and 4,946,778, and EP publications 173,494 and 239,400. Alternatively, one may isolate DNA sequences which code for a human monoclonal antibody or portions thereof that specifically bind to the receptor protein by screening a DNA library from human B cells according to the general protocol outlined in WO 90/14430, and then cloning and amplifying the sequences which encode the antibody (or binding fragment) of the desired specificity.

Usually, polypeptides used for producing antibodies are the full-length receptor or receptor fragments designed from putative extracellular domains identified by a variety of methods known in the art, including computer programs which predict secondary and tertiary structure of a polypeptide based upon its primary amino acid sequence. Another method for designing antigenic peptides utilizes computer programs that predict the high points of hydrophilicity within a particular primary amino acid sequence. For example, using the method of Happ and Woods, *Proc. Natl. Acad. Sci. USA* (1981) 78:3824-3829, via the "Antigen" program in PC/GENE, the inventors identified 3 regions of high hydrophilicity, shown below in Table 7, and used the results to design antigenic peptides to be used in the preparation of antibodies against GBS toxin receptor (see Example 4).

Table 7
High Points of Hydrophilicity in SP55

25

10

15

20

No.	Ah	Sequence
1	2.05	Glu-Glu-Gly-Ser-Asp-Arg (14-19 of SEQ ID No. 2)
2	1.52	Lys-Asp-Asn-Arg-Thr-Ser (75-80 of SEQ ID No. 2)
3	1.33	Arg-Ala-Pro-Arg-Ala-Glu (25-30 of SEQ ID No. 2)

Ah = Average hydrophilicity.

Antibodies that recognize various portions of the intact GBS toxin receptor can be used to further investigate structure and function of the receptor. The polypeptides of the invention can give rise to antibodies that recognize a variety of forms of GBS toxin receptor, including, but not limited to, intact GBS toxin receptor

expressed on a cell surface, denatured GBS toxin receptor or non-denatured GBS toxin receptor, and GBS toxin receptor purified away from cellular components or GBS toxin receptor contained in a cell lysate. GBS toxin receptor antibodies can be used to study species differences as well as GBS toxin receptor expression levels in various cell types.

Antibodies that recognize a portion or all of an extracellular domain are particularly useful as a diagnostic for the monitoring of tumor growth and metastasis, for the detection or identification of a chronic inflammatory condition, such as, for example, rheumatoid arthritis or psoriasis, and for the detection of other medical conditions arising due to hypoxia-driven angiogenesis, such as, for example, restenosis. Typically, such antibodies can be employed in a variety of standard research and diagnostic techniques, including, but not limited to, western blot, immunoprecipitation, ELISA, radioimmunoassay (RIA), BIACOR®, enzyme-linked-immunoassay (EIA), immunofluorescence, fluorescence activated cell sorting (FACS), and *in vivo* diagnostic imaging systems such as magnetic resonance imaging (MRI), nuclear magnetic resonance (NMR), computerized axial tomography (CAT) scan, and position emission tomography (PET), etc.

In addition, antibodies that block the binding of GBS toxin to a GBS toxin receptor can be used for the treatment or prevention of early onset disease in a neonatal human. Such antibodies can directly or indirectly block the GBS toxin binding site on the GBS toxin receptor.

In one embodiment, the GBS toxin receptor protein is naturally occurring and can be isolated from a cell extract by protein purification techniques known in the art, such as, for example, ion exchange column chromatography, high performance liquid chromatography (HPLC), reversed phase HPLC, or affinity chromatography using antibodies that recognize the GBS toxin receptor.

Alternatively, the isolated proteins and polypeptides are expressed using polynucleotides encoding the polypeptide(s) of the invention in operative association with an appropriate control sequence including a promoter in an expression vector suitable for expression, preferably in a mammalian cell, and also in bacterial, insect, or yeast cells.

Usually, the GBS toxin receptor polynucleotide or a fragment thereof can be expressed in a mammalian system. Such expression will usually depend on a mammalian promoter, which is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (e.g. structural gene) into mRNA. Usually, a promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region typically includes an RNA polymerase binding site and a transcription initiation site.

5

10

15

20

25

Vectors suitable for replication in mammalian cells are known in the art, and can include viral replicons, or sequences that ensure integration of the sequence encoding PAK65 into the host genome. Suitable vectors can include, for example, those derived from simian virus SV40, retroviruses, bovine papilloma virus, vaccinia virus, and adenovirus.

A suitable vector, for example, is one derived from vaccinia viruses. In this case, the heterologous DNA is inserted into the vaccinia genome. Techniques for the insertion of foreign DNA into the vaccinia virus genome are known in the art, and utilize, for example, homologous recombination. The insertion of the heterologous DNA is generally into a gene which is non-essential in nature, for example, the thymidine kinase gene (tk), which also provides a selectable marker. Plasmid shuttle vectors that greatly facilitate the construction of recombinant viruses have been described (see, for example, Mackett et al. (1984); Chakrabarti et al. (1985); Moss (1987)). Expression of the heterologous polypeptide then occurs in cells or individuals which are immunized with the live recombinant vaccinia virus.

Such suitable mammalian expression vectors usually contain one or more eukaryotic transcription units that are capable of facilitating expression in mammalian cells. The transcription unit is comprised of at least a promoter element to mediate transcription of foreign DNA sequences. Suitable promoters for mammalian cells are known in the art and include viral promoters such as those from simian virus 40 (SV40) (Subramani et al., Mol Cell. Biol. 1:854–864, 1981), cytomegalovirus (CMV) (Boshart et al., Cell 41:521–530, 1985), Rous sarcoma virus (RSV), adenovirus (ADV) (Kaufman and Sharp, Mol. Cell. Biol. 2:1304–1319, 1982), and bovine papilloma virus (BPV), as well as cellular promoters, such as a mouse metallothionein-1 promoter (U.S. Patent No. 4,579,821), a mouse VK promoter (Bergman et al., Proc. Natl. Acad. Sci. USA 81:7041–7045, 1993; Grant et al., Nuc. Acids Res. 15:5496, 1987), and a mouse VH promoter (Loh et al., Cell 33:85–93, 1983).

The optional presence of an enhancer element (enhancer), combined with the promoter elements described herein, will typically increase expression levels. An enhancer is any regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to endogenous or heterologous promoters, with synthesis beginning at the normal mRNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or

٠5

10

15

20

25

flipped orientation, or at a distance of more than 1000 nucleotides from the promoter (Maniatis et al. (1987) Science 236:1237; Alberts et al. (1989) Molecular Biology of the Cell, 2nd ed.). Enhancer elements derived from viruses can be particularly useful, because they typically have a broader host range. Examples useful in mammalian cells include the SV40 early gene enhancer (Dijkema et al (1985) EMBO J. 4:761) and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus (Gorman et al. (1982b) Proc. Natl. Acad. Sci. 79:6777), from human cytomegalovirus (Boshart et al. (1985) Cell 41:521) as well as the mouse μ enhancer (Gillies, Cell 33:717–728, 1983). Additionally, some enhancers are regulatable and become active only in the presence of an inducer, such as a hormone or metal ion (Sassone-Corsi and Borelli (1986) Trends Genet. 2:215; Maniatis et al. (1987) Science 236:1237).

In addition, the transcription unit can also be comprised of a termination sequence and a polyadenylation signal which are operably linked to the GBS toxin receptor coding sequence. Polyadenylation signals include, but are not limited to, the early or late polyadenylation signals from SV40 (Kaufman and Sharp), the polyadenylation signal from the Adenovirus 5 E1B region and the human growth hormone gene terminator (DeNoto et al., Nuc. Acids Res. 9:3719–3730, 1981).

Sequences that cause amplification of the gene may also be desirable, as are sequences which encode selectable markers. Selectable markers for mammalian cells are known in the art, and include, for example, thymidine kinase, dihydrofolate reductase (together with methotrexate as a DHFR amplifier), aminoglycoside phosphotransferase, hygromycin B phosphotransferase, asparagine synthetase, adenosine deaminase, and antibiotic resistant genes such as neomycin.

A GBS toxin receptor, or fragment thereof, can be expressed on the surface of a cell, or can be expressed in soluble or secreted form. Expression on the surface of the cell can be achieved, for example, by including a secretory leader operably linked to a nucleic acid sequence encoding the desired receptor fragment and at least one transmembrane domain. The secretory leader can be that encoded by the GBS toxin receptor gene, or can be a heterologous leader sequence commonly used in the art, such as, for example, the leader sequence of Schizosaccharomyces pombe pho1+ acid phosphatase (Braspenning et al., Biochem Biophys Res. Commun (1998) 245:166-71), the leader sequence of human interleukin-2 (IL-2) gene (Sasada et al., Cell Struct

10

15

20

25

Funct (1988) 13:129-141). Expression in soluble or secreted form can be achieved. for example, by excluding from the gene construct nucleic acid sequences encoding a transmembrane domain. In some instances, solubility and/or secretion are achieved by the use of a fusion partner, such as, for example, chloramphenicol acetyltransferase (CAT), β-galactosidase, and other genes readily expressed in the selected host cell.

The vector that encodes GBS toxin receptor can be used for transformation of a suitable mammalian host cell. Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus and transducing a host cell with the virus or by transfection procedures known in the art, as exemplified by U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (these patents are incorporated herein by reference). The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), N1E-115 (Liles et al., J. Biol. Chem. 261:5307–5313, 1986), PC 12 human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines, such as insect derived cell lines IF9 and IF21. Cell lines of particular preference are those expressing recombinant GBS toxin receptor constructs constitutively, lines which subsequently develop characteristics of a transformed cell, and lines which more preferably express GBS toxin receptor or fragments on the cell surface. Particularly preferred are ECV cells (a bladder carcinoma cell line originally referred to in the scientific literature as an endothelial cell line), human umbilical vein endothelial cells (HUVEC), bovine, sheep, and human adrenal medulla endothelial cells.

Recombinant GBS toxin receptor or fragments thereof can be produced by culturing host cells expressing the receptor or fragment in a suitable culture medium and under appropriate cell culture conditions. Culture media and conditions are

10

15

20

25

variable depending on the requirements of a particular host cell line and are well known in the art. Typically, cells are cultured at 37°C in a cell culture incubator with a fixed amount of C02, usually in the range of 5-10%.

In another embodiment, the polypeptide fragments can be synthesized chemically by techniques well known in the art, such as solid-phase peptide synthesis (Stewart et al., Solid Phase Peptide Synthesis, W.H. Freeman Co., San Francisco (1963)); Merrifield, J Am Chem Soc 85:2149-2154 (1963)). These and other methods of peptide synthesis are also exemplified by U.S. Patent Nos. 3,862,925, 3,842,067, 3,972,859, and 4,105,602. The synthesis can use manual synthesis techniques or automatically employ, for example, an Applied BioSystems 430A or 431A Peptide Synthesizer (Foster City, California) following the instructions provided in the instruction manual supplied by the manufacturer. It will be readily appreciated by those having ordinary skill in the art of peptide synthesis that the intermediates which are constructed during the course of synthesizing the present analog compounds are themselves novel and useful compounds and are thus within the scope of the invention.

In addition to polypeptides consisting only of naturally-occurring amino acids. peptidomimetics are also provided. Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compounds are termed "peptide mimetics" or "peptidomimetics" (Fauchere, J. (1986) Adv. Drug Res. 15: 29; Veber and Freidinger (1985) TINS p.392; and Evans et al. (1987) J. Med. Chem 30: 1229, which are incorporated herein by reference) and are usually developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biochemical property or pharmacological activity) but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: -CH2NH-, -CH2S-, -CH2-CH2-, -CH=CH-(cis and trans), -COCH2-, -CH(OH)CH2-, and -CH2SO-, by methods known in the art and further described in the following references: Spatola, A.F. in "Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins," B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983); Vol. 1, Issue 3, "Peptide Backbone Modifications" (general review); Morley, J.S., Trends Pharm Sci (1980) pp. 463-468 (general

5

10

15

20

25

review); Hudson, D. et al., Int J Pept Prot Res (1979) 14:177-185 (-CH2NH-, CH2CH2-); Spatola, A.F. et al., Life Sci (1986) 38:1243-1249 (-CH2-S); Hann, M.M., J Chem Soc Perkin Trans I (1982) 307-314 (-CH-CH-, cis and trans): Almquist, R.G. et al., J. Med Chem (1980) 23:1392-1398 (-COCH2-); Jennings-White, C. et al., Tetrahedron Lett (1982) 23:2533 (-COCH2-); Szelke, M. et al., 5 European Appln. EP 45665 (1982) CA: 97:39405 (1982) (-CH(OH)CH2-); Holladay, M.W. et al., Tetrahedron Lett (1983) 24:4401-4404 (-C(OH)CH2-); and Hruby, V.J., Life Sci (1982) 31:189-199 (-CH2-S-); each of which is incorporated herein by reference. A particularly preferred non-peptide linkage is -CH2NH-. Such peptide 10 mimetics may have significant advantages over polypeptide embodiments, including, for example: more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (e.g., a broad-spectrum of biological activities), reduced antigenicity, and others. Labeling of peptidomimetics usually involves covalent attachment of one or more labels, directly or through a spacer (e.g., an amide group), to non-interfering 15 position(s) on the peptidomimetic that are predicted by quantitative structure-activity data and/or molecular modeling. Such non-interfering positions generally are positions that do not form direct contacts with GBS toxin (e.g., are not contact points in the GBS toxin binding domain of the GBS toxin receptor). Derivitization (e.g., labelling) of peptidomimetics should not substantially interfere with the desired 20 biological or pharmacological activity of the peptidomimetic.

Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch (1992) Ann. Rev. Biochem. 61: 387, incorporated herein by reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

The invention also provides a complex comprising a GBS toxin bound to a mammalian GBS toxin receptor or a fragment of a mammalian GBS toxin receptor. Preferably, the complex comprises a GBS toxin bound to a GBS toxin receptor polypeptide described above that can bind GBS toxin. Typically, a complex is formed by contacting a GBS toxin with such a polypeptide under conditions that

25

permit specific binding of the GBS toxin to the polypeptide. The GBS toxin can be labeled or unlabeled. The polypeptide can be present on the surface of a cell, or immobilized in a well or on a bead, or the polypeptide can be present in solution.

5 DETECTION METHODS

10

15

20

25

30

Yet another aspect of the invention provides methods for detecting or monitoring a variety of medical conditions characterized by pathologic and/or hypoxia-driven angiogenesis or neovascularization. Examples include, but are not limited to, early onset disease in the neonate, and the progression of cancers involving tumors.

Early onset disease can be diagnosed by detecting the presence or absence of GBS toxin in a patient. One method of detection is a competition assay that determines the effect of a suspected sample on the formation of a complex between GBS toxin and a GBS toxin receptor or fragment thereof. For example, the method comprises contacting a control GBS toxin with a GBS toxin receptor polypeptide, in the presence and absence of a sample suspected of containing GBS toxin and under conditions that permit specific binding of the GBS toxin to the polypeptide, and comparing the amount of complex formation achieved in the presence of the suspected sample to the amount of complex formation achieved in the absence of the suspected sample. Preferably, the control GBS toxin is substantially purified and of a known concentration. Preferably, the control GBS toxin further comprises a label. Suitable labels include, but are not limited to, radioisotopes, chromophores, fluorophores, biotin, avidin, and other labels used by one skilled in the art. Another method directly measures, rather than by competition with a control GBS toxin, complex formation between GBS toxin present in a suspected sample and a GBS toxin receptor polypeptide.

Pathologic vasculature can be detected in a mammalian tissue by detecting the presence or absence of GBS toxin receptor in the region of a tumor, with the presence of GBS toxin receptor being indicative of the presence of pathologic vasculature. The method can be used to monitor tumor growth or metastasis. One method of detection involves the use of molecules, e.g. antibodies, that specifically bind to a GBS toxin receptor, preferably an extracellular domain of GBS toxin receptor. Typically, the method comprises administering, to a mammalian tissue, e.g. in a mammal having a cancerous tumor, e.g., an antibody that recognizes a GBS toxin receptor, and

detecting specific binding of the antibody. Typically, the antibody is a labeled antibody. Preferably, the observations are quantitative and can be visual.

During surgery, the margin of a tumor can be visualized by any of a number of imaging techniques known in the art and described above. The imaging of the tumor is effected by detecting the binding of a labeled antibody or other molecules to the GBS toxin receptor on the pathologic vasculature of a tumor. This type of surgery is also known as virtual surgery because while performing the surgery, the surgeon views the tumor indirectly on an imaging screen.

10 DRUG DISCOVERY

5

15

20

25

30

A fourth aspect of the invention provides methods, using the polypeptides of the invention, of identifying drug candidates for the treatment of medical conditions characterized by hypoxia-driven angiogenesis or neovascularization. Preferred compounds are competitive inhibitors of GBS toxin binding to a GBS toxin receptor or inhibit GBS toxin receptor activity. Particularly preferred are compounds that inhibit the first phosphorylation step in the signal transduction pathway. Compounds can be produced by a variety of random drug design methods commonly known in the art, such as, for example, combinatorial chemistry (U.S. Patent No. 5,646,285; U.S. Patent No. 5,639,603), peptide libraries (U.S. Patent No. 5,591,646; U.S. Patent No. 5,367,053; U.S. Patent No. 5,747,334), phage display (U.S. Patent No. 5,403,484; U.S. Patent No. 5,223,409), SELEX® (U.S. Patent No. 5,773,598; U.S. Patent No. 5,763,595; U.S. Patent No. 5,763,566), and combinatorial carbohydrate chemistry (Hirschmann et al., J Med Chem (1996) 39:2441-2448; Hirschmann et al., J Med Chem (1998) 41:1382-1391; Sofia MJ, Mol Divers (1998) 3:75-94; U.S. Patent No. 5,780,603; U.S. Patent No. 5,756,712)

An alternative approach is rational drug design with the intent of producing a GBS toxin mimetic or a GBS toxin receptor mimetic with improved therapeutic properties using techniques such as x-ray crystallography, nuclear magnetic resonance (NMR) correlation spectra (U.S. Patent No. 5,698,401), computer assisted molecular modeling (U.S. Patent No. 5,579, 250; U.S. Patent No. 5,612,895; U.S. Patent No. 5,680,331, Cooper et al., J. Comput.-Aided Mol. Design, 3:253-259 (1989); Brent et al., J. Comput.-Aided Mol. Design 2:311-310 (1988)) and other methods of rational drug design known in the art. **FIG. 1** provides a broad overview of some of the main steps in some of the rational drug design methods of the present invention. For

example, one approach to rational drug design involves a computer program, such as INSIGHTII (available from Bisoym Technologies, 10065 Barnes Canyon Road, San Diego, California) to identify active sites in proteins by homology-based modeling. This method facilitates the modeling of a protein by using a similar protein whose structure is well known. Commercial software containing search algorithms for three dimensional database comparisons are available from vendors such as Day Light Information Systems, Inc., Irvine, California 92714, and Molecular Design Limited, 2132 Faralton Drive, San Leandro, California 94577.

In one embodiment, the compound can bind the GBS toxin receptor and induce an inflammatory response in a manner similar to the binding of GBS toxin to the GBS toxin receptor. Such compounds can be used, for example, as a drug to target an inflammatory response to the developing vasculature of a tumor.

In another embodiment, the compound can bind the GBS toxin receptor with or without inducing an inflammatory response, preferably without inducing an inflammatory response. In one instance, the compound can be used as a vehicle to target pathological neovasculature for treatment with a cytotoxic agent. For example, the cytotoxic agent can be chemically coupled to the compound to form a chimeric drug. Such chimeric drugs can be used in the treatment of tumors, rheumatoid arthritis, wound healing, spinal cord injury, and other conditions characterized by hypoxia-driven angiogenesis or neovascularization. In another instance, the compound can be used directly to competitively inhibit binding of GBS toxin to a GBS toxin receptor. Such compounds can be used in the treatment of early-onset disease in the neonate.

In a third embodiment, the compound can bind GBS toxin and can be used in the treatment of early-onset disease in the neonate.

The polynucleotides of the invention can be expressed in random mutagenesis systems such as phage display or the yeast two-hybrid system for the synthesis and identification of mutant peptide GBS toxin receptor polypeptides that bind GBS toxin. Alternatively, immobilized or soluble GBS toxin receptor fragments of the invention can be used to screen combinatorial peptide and combinatorial chemical libraries and non-random recombinant and synthetic peptides and other compounds (such as non-peptide molecules) for GBS toxin receptor binding. Compounds that bind GBS toxin or GBS toxin receptor can then be further characterized in a functional assay for any of the activities described above in order to identify a drug candidate for the treatment

5

10

15

20

25

of medical conditions involving angiogenesis or neovascularization.

A compound which inhibits binding of GBS toxin to a GBS toxin receptor can be identified by combining a test compound with a mammalian GBS toxin receptor or fragment thereof capable of binding GBS toxin, under conditions that permit specific binding of GBS toxin to the GBS toxin receptor or fragment, and determining the amount of inhibition by the compound of the binding of GBS toxin to the GBS toxin receptor or fragment.

In a preferred embodiment, the GBS toxin receptor or fragment is expressed by a cell, preferably on the cell surface. The cells are contacted with labeled GBS toxin in the presence or absence of the test compound. A change in the binding of GBS toxin to the GBS toxin receptor is then determined. Alternatively, the GBS toxin is unlabeled and an antibody that recognizes GBS toxin is labeled instead. The labeled antibody is used to measure inhibition by a compound of GBS toxin binding to the GBS toxin receptor or fragment. In another embodiment, the GBS toxin receptor or fragment is not associated with a cell, but is instead coupled to a matrix, such as, for example, a well in a microtiter plate or a bead. Additional suitable solid supports include latex, polystyrene beads (Interfacial Dynamics Corp. Portland, Oreg.), magnetic particles (Advanced Magnetics, Cambridge, Mass.) and nylon balls (Hendry et al., J. Immunological Meth., 35:285-296, 1980). The receptor or fragment can be coupled to the matrix directly or indirectly through an antibody, coupled to the matrix, that binds the receptor fragment. In a third embodiment, the GBS toxin receptor or fragment is soluble and can be immunoprecipitated with an antibody that recognizes the receptor or fragment.

A preferred method for identifying a compound which binds a mammalian GBS toxin receptor comprises the steps of (1) combining a test compound with a GBS toxin receptor or fragment thereof under conditions that allow specific binding to occur, and (2) detecting a complex formed between the test compound and the GBS toxin receptor or fragment. A preferred method is a competition assay which determines the ability of the test compound to compete for binding to the GBS toxin receptor or fragment. In such an assay, GBS toxin is combined with the GBS toxin receptor or fragment in the presence or absence of the test compound. Decreased specific binding of GBS toxin in the presence versus the absence of the test compound is indicative of the ability of the test compound to bind a mammalian GBS toxin receptor. Another method comprises combining a control compound with the GBS

5

10

15

20

25

toxin receptor or fragment under the same conditions as the test compound and comparing the amount of complex formation between the test compound or the control compound and the GBS toxin receptor or fragment thereof. Preferably, the test compound and/or the control compound are labeled. The test compound can be any of a number of classes of compounds, such as for example, small organic molecules (such as those used for and obtained by combinatorial chemistry), polysaccharides, polypeptides, RNA, antibodies, and single chain antibodies. In a preferred embodiment, the polypeptide is expressed by a cell, preferably on the surface of the cell, and preferably by a stable transfected cell. Such a system is particularly useful for testing the effectiveness of a chimeric compound comprising a cytotoxic agent. The cytotoxic activity of the compound can be determined by exposing a cell expressing the GBS toxin receptor on the cell surface to the test chimeric compound and detecting signs of cytotoxicity. One could detect such signs by a viability stain of the cell, by detecting apoptosis (for example, by a DNA ladder assay or a TUNELTM stain, which binds to broken DNA), by measuring tritiated thymidine incorporation into the cell, and by quantitating kinase-dependent phosphorylation (e.g., using phosphoantibodies or various phosphoimaging techniques).

In another embodiment, the invention provides a method for identifying an inhibitor of GBS toxin receptor. The method comprises incubating test cells in the presence and absence of a test compound. The test cells express GBS toxin receptor or a fragment thereof having GBS toxin receptor activity (e.g., a fragment that increases the proliferation or migration of the expressing cells relative to control cells of the same cell type that do not express the fragment). The test cells are incubated under conditions in which the cells incubated in the absence of the test compound can proliferate or migrate. Control cells that do not express the GBS toxin receptor or fragment proliferate or migrate less than cells that express the GBS toxin receptor or fragment. The proliferation or migration (also referred to herein as motility) of the test cells incubated in the presence or absence of the test compound is compared. Less proliferation or migration in the presence of the test compound than in the absence of the test compound is indicative of the test compound being an inhibitor of the GBS toxin receptor. Preferably, as a control to determine whether the test compound specifically inhibits the GBS toxin receptor, the proliferation or migration of control cells in the presence and absence of the test compound is also compared. In the

5

10

15

20

25

absence of a difference in the proliferation or migration of control cells incubated in the presence or absence of the test compound, decreased proliferation or migration in test cells exposed to the test compound relative to test cells not exposed to the test compound is indicative of specific inhibition of the GBS toxin receptor. It will be readily apparent that the control portions of the method need not be performed contemporaneously with the test portions of the method. For example, control cells can be incubated with a battery of test compounds to determine cellular effects of the test compounds prior to incubating the test cells with the test compounds. Motility or migration can be determined by detecting movement of cells on a culture dish. Proliferation can be detected in a number of ways, including, but not limited to,

measuring tritiated thymidine incorporation, cell counts, apoptosis assays, and viability assays. Preferred cells include cells transfected with GBS toxin receptor, preferably endothelial cells transfected with GBS toxin receptor, even more preferably vascular endothelial cells or microvascular endothelial cells. Primary cells that express GBS toxin receptor are also preferred, for example, endothelial cells that have been passaged in cell culture, at confluence, no more than 8 or 9 times. A preferred class of test compounds includes kinase inhibitors, preferably cAMP-dependent kinase inhibitors, PKC inhibitors, and CK2 inhibitors, which can be used as a starting point for developing more specific GBS toxin receptor inhibitors.

Another class of compounds includes antibodies specific for GBS toxin receptor. Particularly preferred are single chain antibodies, preferably a collection of single chain antibodies that recognize various epitopes on the GBS toxin receptor. Less preferred are divalent antibodies specific for the binding site of the GBS toxin receptor ligand because they may trigger the signal transduction cascade upon dimerization.

Another embodiment of the invention is a method of identifying an inhibitor of endothelial cell proliferation or migration, which are essential components of angiogenesis. The method basically comprises the steps described in the preceding paragraph and uses endothelial cells.

Yet another embodiment of the invention is a method of identifying a therapeutic compound for the treatment or prevention of a medical condition characterized by pathologic or hypoxia-driven angiogenesis or neovascularization. The method basically comprises the steps described above and uses cells from tissues derived from mammals afflicted with the medical condition or cells that serve as a

5

10

15

20

25

model for afflicted tissue.

5

10

15

20

A preferred method for designing a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor comprises (1) simulating and selecting the most probable conformations of a GBS toxin receptor or fragment thereof, (2) designing a chemically modified analog that substantially mimics the energetically most probable three-dimensional structure of the GBS toxin receptor or fragment, (3) chemically synthesizing the analog, and (4) evaluating the bioactivity of the analog. Preferably, steps (a) and (b) are performed with the aid of a computer program.

A preferred method for designing a compound which binds to a mammalian GBS toxin receptor comprises (1) simulating and selecting the most probable conformations of a GBS toxin receptor or fragment thereof, (2) deducing most probable binding domains of the receptor or fragment, (3) designing a compound that would form the energetically most probable complexes with the receptor or fragment, (4) chemically synthesizing the compound, and (5) evaluating the bioactivity of the compound. Preferably, steps (a)-(c) are performed with the aid of a computer program.

Preferred polypeptides for use in the screening assays described above are polypeptides sharing at least about 85% identity, preferably at least about 95% identity, and most preferably greater than about 99% identity with the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4, or a fragment thereof having GBS toxin receptor activity. Most preferred are polypeptides having an amino acid sequence of SED ID NO: 2, 4 OR 8 or a fragment thereof having GBS toxin receptor activity.

25 METHODS OF PURIFICATION

Another aspect of the invention is a method for purifying a compound that binds a GBS toxin receptor, for example, natural ligand, other polysaccharides, or an antibody specific for the GBS toxin receptor. The method comprises providing a polypeptide comprising a mammalian GBS toxin receptor or fragment thereof that binds GBS toxin, contacting the polypeptide with a sample comprising the compound under conditions that allow specific binding of the compound to the polypeptide, and separating the bound compound from the remainder of the sample. The polypeptide can be soluble but preferably is immobilized on a substrate e.g., on a bead, membrane or on the surface of a cell, preferably a stable transfected cell.

METHODS OF TREATMENT

5

GBS toxin receptor polypeptides and antibodies that interfere with GBS toxin binding can be used in a method of treatment of the human or animal body. For example, such inhibitors of GBS toxin binding can be administered to a patient to treat or prevent medical conditions involving GBS toxin binding to a GBS toxin receptor, such as, for example, early onset disease in the neonate.

GBS toxin mimetics or other compounds that bind and/or inhibit GBS toxin receptor, some of which can be identified by the drug discovery assays of the invention, can be used in a method of treatment of the human or animal body or can be used for the manufacture of a medicament for the treatment or prevention of any of a number of medical conditions involving pathologic and/or hypoxia-driven angiogenesis, such as, for example, cancerous tumors, chronic inflammatory diseases, scarring during wound healing or repair of neural injury.

In a preferred embodiment, such a compound exerts its therapeutic effect by binding GBS toxin receptor and evoking an inflammatory response, as does GBS toxin. Preferably, such compounds comprise a sulfhydryl, hydroxyl, or amino group displayed so as to be available for binding complement C3.

In another preferred embodiment, the compound is an inhibitor of GBS toxin activity. Preferred inhibitors include, but are not limited to, kinase inhibitors, single chain antibodies specific for the GBS toxin receptor, and antisense polynucleotides that specifically hybridize under high stringency conditions to a GBS toxin receptor nucleic acid sequence, such as that of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:7.

In another preferred embodiment, the compound exerts its therapeutic effect

without evoking an inflammatory response. The compound can be used to deliver a cytotoxic agent to tissue in close proximity to a cell expressing a GBS toxin receptor, such as, for example, a tumor undergoing angiogenesis. Preferably, the compound is covalently attached to a cytotoxic agent and can be associated non-covalently with a cytotoxic agent, such as, for example, on the external surface of a liposome, micelle, or other lipophilic drug encapsulating structure. Preferred cytotoxic agents include antineoplastic agents commonly known in the art, such as, for example. mechlorethamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide, and other alkylating agents, methotrexate and other folate antagonists, 6-mercaptopurine and other purine antagonists, 5-fluorouracil and other pyrimidine antagonists, cytarabine, ovinblastine, vincustine, and other vincas, etoposide and other podophyllotoxins, doxorubicin, bleomycin, mitomycin, and other antibiotics, carmustine, lomustine and other nitrosureas, cisplatin, interferon, asparaginase, tamoxifen, flutamide, and taxol. Other preferred biologic agents include sense and/or antisense RNA or DNA sequences derived from specific tumor promoter or suppressor genes, such as, for example, the p53 and TGF gene families, signal transduction protein family members such as, for example, ras and myc, and growth factor receptor kinases such as, for

10

15

20

25

example flt2 and flk1, Tai1, Tai2, and neuropholin, and other genes implicated in neoplastic disease and other diseases driven by pathologic angiogenesis.

In another embodiment, GBS toxin receptor polypeptide or fragment thereof can be administered to a subject as a decoy to reduce the amount of stimulation of the GBS toxin receptor present in afflicted tissues (e.g., tumor tissues), thereby reducing cellular responses leading to proliferation and migration of cells of the afflicted tissues. Preferably, the GBS toxin receptor polypeptide or fragment is administered in soluble form, even more preferably sans transmembrane domains.

10 PHARMACEUTICAL COMPOSITIONS

Polypeptides of the invention that comprise a domain essential for GBS toxin binding that have the desired characteristics for bioavailability, stability and other important parameters of pharmacokinetics *in vivo* can be used as a competitive inhibitor of GBS toxin binding for medical conditions, such as, for example, early onset disease in the neonate, in which GBS toxin binding is undesirable. Appropriate polypeptides can include fragments having an amino acid sequence corresponding to a partial or full sequence of SEQ ID NO: 2 or SEQ ID NO: 4 or analogs thereof.

Compounds determined by assays using the polypeptides of the invention to bind and/or GBS toxin receptor and/or induce an inflammatory response, and that have the desired pharmacokinetic characteristics, can be used as treatments for medical conditions in which GBS toxin binding can be therapeutic, such as, for example, medical conditions involving pathologic or hypoxia-driven angiogenesis or neovascularization.

Pharmaceutical compositions of the invention include a pharmaceutically acceptable carrier that may contain a variety of components that provide a variety of functions, including regulation of drug concentration, regulation of solubility, chemical stabilization, regulation of viscosity, absorption enhancement, regulation of pH, and the like. For example, in water soluble formulations the pharmaceutical composition preferably includes a buffer such as a phosphate buffer, or other organic acid salt, preferably at a pH of between about 7 and 8. Other components may include antioxidants, such as ascorbic acid, hydrophilic polymers, such as, monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, dextrins, chelating agents, such as EDTA, and like components well

15

20

25

known to those in the pharmaceutical sciences, e.g. Remington's Pharmaceutical Science, latest edition (Mack Publishing Company, Easton, PA).

An effective amount of an active compound such as a GBS toxin receptor polypeptide, mimetic or analog, or GBS toxin mimetic or analog for particular applications depends on several factors, including the chemical nature of the polypeptide, mimetic or analog, the disorder being treated, the method of administration, and the like. Preferably, an effective amount will provide a concentration of polypeptide or mimetic of between about 0.0001 to 100 μ M at the target GBS toxin receptor on a cell surface, more preferably less than 10 μ M, with less than 1 μ M being most preferred.

The active compound can be administered to a mammalian host in a variety of forms, i.e., they may be combined with various pharmaceutically acceptable inert carriers in the form of tablets, capsules, lozenges, troches, hard candies, powders, sprays, elixirs, syrups, injectable or eye drop solutions, and the like depending on the chosen route of administration, e.g., orally or parenterally. Parenteral administration in this respect includes administration by the following routes: intravenous, intramuscular, subcutaneous, intraocular, intrasynovial, transepithelial (including transdermal, ophthalmic, sublingual and buccal), topical (including ophthalmic, dermal, ocular, rectal, nasal inhalation via insufflation and aerosol), and rectal systemic.

The active compound may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, it may be enclosed in hard or soft shell gelatin capsules, compressed into tablets, or incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at lease 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2% to about 6% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 1 and 1000 mg of active compound.

5

10

15

20

25

Tablets, troches, pills, capsules and the like may also contain the following: a binder such as polyvinylpyrrolidone, gum tragacanth, acacia, sucrose, com starch or gelatin; an excipient such as calcium phosphate, sodium citrate and calcium carbonate; a disintegrating agent such as corn starch, potato starch, tapioca starch. certain complex silicates, alginic acid and the like; a lubricant such as sodium lauryl sulfate, talc and magnesium stearate; a sweetening agent such as sucrose, lactose or saccharin; or a flavoring agent such as peppermint, oil of wintergreen or cherry flavoring. Solid compositions of a similar type are also employed as fillers in soft and hard-filled gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye, flavoring such as cherry or orange flavor, emulsifying agents and/or suspending agents, as well as such diluents as water, ethanol, propylene glycol, glycerin and various combinations thereof. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

The active compound may also be administered parenterally or intraperitoneally. For purposes of parenteral administration, solutions in sesame or peanut oil or in aqueous propylene glycol can be employed, as well as sterile aqueous solutions of the corresponding water-soluble, alkali metal or alkaline-earth metal salts previously enumerated. Such aqueous solutions should be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. Solutions of the active compound as a free base or a pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. A dispersion can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal injection purposes. In

5

10

15

20

25

this connection, the sterile aqueous media employed are all readily obtainable by standard techniques well-known to those skilled in the art.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of a dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid. thimerosal and the like. In many cases it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by use of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying technique which yield a powder of the active ingredient plus any additional desired ingredient from the previously sterile-filtered solution thereof.

For purposes of topical administration, dilute sterile, aqueous solutions (usually in about 0.1% to 5% concentration), otherwise similar to the above parenteral solutions, are prepared in containers suitable for drop-wise administration to the eye. The compounds of this invention may be administered to a mammal alone or in combination with pharmaceutically acceptable carriers. As noted above, the relative proportions of active ingredient and carrier are determined by the solubility and

5

10

15

20

25

chemical nature of the compound, chosen route of administration, the particular compound chosen and the physiological characteristics of the particular patient under treatment.

5 Kits

10

15

Yet another aspect of the invention is a kit for use in carrying out any of the above methods. A preferred embodiment is a kit comprising a GBS toxin receptor or fragment thereof. Preferably, the receptor or fragment is immobilized. A preferred kit can be used for identifying a compound that binds to GBS toxin receptor, and comprises at least one cell that expresses GBS toxin receptor.

Another embodiment is a kit for monitoring tumor growth or metastasis, comprising a reagent for detecting expression of a GBS toxin receptor. Examples of such reagents include, but are not limited to, polynucleotide probes that hybridize to a GBS toxin receptor nucleic acid sequence and compounds that bind to a GBS toxin receptor, such as, for example, an antibody that specifically recognizes GBS toxin receptor, a GBS toxin, a GBS toxin mimetic, or other compounds identified by the screening methods described above.

A third embodiment is a kit for purifying a compound that binds a GBS toxin receptor, comprising a GBS toxin receptor or fragment thereof that binds the compound. Preferred compounds include GBS toxin, GBS toxin mimetics, antibodies that specifically bind GBS toxin receptor, and other compounds identified by the screening methods described above.

Additional kit components can include, but are not limited to, additional reagents required for detection, a reference standard(s), instructions for use, and the like. Suitable reference standards include positive controls, negative controls, photographs of such controls, tabulated or graphed data of such controls, and the like. The kits may further comprise instructions for carrying out the methods described above, preferably printed instructions.

EXAMPLES

EXAMPLE 1 - CLONING SHEEP GBS TOXIN RECEPTOR

Primary culture of sheep lung endothelial cells

Small pieces of primary lung tissues from a 7-week old sheep are cut into small pieces in Hank's balanced salt solution (HBSS) containing 10 mM HEPES buffer (Life Technology), 1% penicillin/streptomycin and 0.1% gentamycin, and are cultured in sheep lung complete medium (Life Technology) at 37°C. After one week of the culture, clones of sheep lung endothelial cells are identified by Cobblestone morphology and harvested into 24-well tissue culture plates (Falcon) using cloning rings. When the cells are confluent, they are detached by pancreatin and transferred to a 60-mm tissue culture Petri dish or a T-25 tissue culture flask (Falcon). When they are confluent again, they are split and cultured into a few 100-mm tissue culture plates (Falcon). Each split is considered to be one passage. The same procedure is repeated until enough cells (~10⁸) are obtained for isolation of mRNA.

Isolation of mRNA and construction of cDNA library

Poly(A)+ RNA is isolated from 9.2 x 10⁷ sheep lung endothelial cells (passage 8 and 9) by a standard method (Pharmacia). A total of 16 μg poly(A)⁺ RNA is acceptable amount obtained. 2.5 μg mRNA can be used to construct a cDNA library. Poly(A)⁺ RNA is oligo(dT)-primed (with *Not* I restriction site) and converted into double-stranded cDNA. After adding a *BstX I/EcoR* I adaptor, the cDNA is unidirectionally cloned into the *BstX* I and *Not* I sites of pCDNA3.1(+) (Invitrogen).

5

10

15

20

25

E. coli Top10F' (Invitrogen) is used as a host strain for amplification. 5.38 x 10⁶ primary clones are an acceptable number generated. The library is amplified by plating cells onto fifty large LB agar plates containing ampicillin (100 μg/ml). The plates are scraped and aliquoted so that each aliquot represents 10 plates. DNA is purified by Qiagen Max columns (Qiagen).

Screening of cDNA library for a gene encoding GBS toxin receptor

To screen a cDNA library for a gene encoding GBS toxin receptor
gene, a unique colorimetric method is used. Five µg plasmid DNA from each pool of
cDNA library is used to transfect COS7 cells. The transfected cells are cultured in
four to eight 96-well tissue culture plates (Falcon) for transient expression. Each well
contains about 20,000 transfected cells in DMEM medium (Life Technology). COS7
cells transfected with pCDNA3.1(+) are used as a control. After 3 days expression,
the medium is carefully removed. Each well is rinsed 3 times with HPSS buffer
containing Mg²⁺ and Ca²⁺ (wash buffer) (Life Technology).

The cells are then incubated with biotinylated toxin ($50\mu l$ per well; 1 to 1.5 $\mu g/ml$) at room temperature for 1 h. After the hour incubation, the biotinylated toxin is discarded and the wells are rinsed 3 times with the wash buffer. The cells are incubated with streptavidin- β -gal solution and each well is rinsed 3 times with the wash buffer. The cells are then incubated with PNPG ($50 \mu l$ per well; 1 mg/ml in substrate buffer) at 37°C. Absorbance at 405 nm is measured by an ELISA reader at 1 and 20 h, respectively. The cells which give the highest OD are harvested. Plasmid DNA is isolated by Hirt extraction. Plasmid DNA is amplified in *E. coli* to have enough DNA for the next transfection (enrichment).

Enrichment is done 8 times by this colorimetric method. The number of the transfected cells loaded into each well is gradually decreased in the last few enrichments and untransfected cells are added to each well to give a total number of 20,000 cells per well for the cells to be confluent and to reduce background after 3 days' expression. At the last enrichment, each well has only 1 to 10 transfected cells. Cells giving the highest OD are harvested. DNA is isolated and amplified in *E. coli*.

A number of isolated clones are individually assayed by this colorimetric method. The clones which showed higher binding to CM101 are sequenced.

Sequence analysis

5

10

15

20

25

DNA sequence analysis of clone pFU102, which has a 2.1kb insert, revealed a sequence encoding a partial integral glycoprotein. N-terminal sequence was obtained by 5'RACE method (Life Technology) and a full-length gene is designated as SP55. Triple ligation yielded pCD55, which contains an entire coding region of SP55.

mRNA for the SP55 has 2844 nucleotides, encoding a protein of 495 amino acids with a predicated mass of 55 KDa, SP55. Analysis by the method of Klein et al. (Klein et al., *Biochim Biophys Acta*, 815:468-476 (1985)) classifies SP55 as an integral protein with seven transmembrane segments. SP55 has both N-glycosylation and kinase phosphorylation sites. A Swiss-Prot. search of SP55 did not reveal any high homology to known human proteins. However, SP55 has some identity (~ 30%) to renal sodium-dependent phosphate transporters from human, rabbit, mouse and rat. In addition, SP55 has some identity (~ 30 to 39%) to hypothetical proteins (HYP50 and HYP63) from *C. elegans*.

15

20

25

30

10

5

EXAMPLE 2 - CLONING HUMAN GBS TOXIN RECEPTOR

The sheep GBS toxin receptor sequence shares about 37% identity with HYP50 and about 33% identity to HYP63, two hypothetical proteins from *C. elegans*. In the regions corresponding to amino acid residues 180-186 and 443-449 of SEQ ID No. 2, five amino acids within a seven amino acid stretch are absolutely conserved among the three proteins.

A first degenerate oligonucleotide, CMR3-S: 5'-CGGGATCCCGCCNGCNATGCAYRSHRTSTGG-3'(SEQ ID No. 5), was designed to include all possible codons encoding the amino acid sequences of SP55, HYP50, and HYP63 in the 180-186 region. A second degenerate oligonucleotide, CMR4-AS2: 5'-GGAATTCCDGGDGCRATKTCNARRTRRTT-3' (SEQ ID No. 6), was designed to include the complementary sequences of all possible codons encoding the amino acid sequences of SP55, HYP50, and HYP63 in the 443-449 region.

Polymerase chain reaction (PCR) was conducted using these oligonucleotides and a human embryo lung cDNA library as a template. The reaction yielded three overlapping sequences approximately 400 bp in size, which encompass part of the nucleic acid sequence of SEQ ID No. 3. These sequences were then used as probes to clone the remainder of the gene, referred to herein and HP59 (SEQ ID NO: 7).

EXAMPLE 3 - PREPARATION OF ANTIBODIES AGAINST GBS TOXIN RECEPTOR

Rabbits are immunized with the synthetic peptides shown in Table 8.

A 1-mg/ml solution of peptide plus KLH in 0.01M phosphate buffer is prepared. For the first immunization, 200 µg of peptide plus KLH (200 µl) and an equal volume of Freunds complete adjuvant, emulsified well before injection, is injected into 3-4 spots on the dorsal surface about the neck and shoulders of a rabbit. After two weeks, the second immunization (boost) is given at the same concentration of immunogen, but emulsified in Freunds incomplete adjuvant. The boost is delivered in the same region of the body. After another two weeks, blood is collected and assayed by ELISA for response against the peptide without KLH. Further boosts are given to improve antibody titer, if necessary.

Table 8

Immunogenic Peptides

<u>Peptide</u>	Amino Acid Sequence	<u>Size</u>	SEQ ID Ref.
p56a	APSDGEEGSDRTPLLQRAPRAEPAPVC	27 aa	residues 8-35 of SEQ ID NO:4
p55a	LAPSDGEEGSDRTPL	15 aa	residues 7-22 of SEQ ID NO: 4
p57a	NTTAKDNRTSYECA	14 aa	residues 71-84 of SEQ ID NO: 4

Peptide p55 is a fragment of an extracellular domain of GBS toxin receptor. Peptide p57a is a fragment of an intracellular domain of GBS toxin receptor. Animals immunized with these peptides produce polyclonal antibodies Pab55 and Pab57, respectively.

Example 4 - Detection of GBS Toxin Receptor Expression in Tumor Cells

This example shows that GBS toxin receptor can be detected in tumor cells.

Immunohistochemistry is performed on paired human and mouse tissues of normal or tumor origin, using rabbit polyclonal antibodies Pab 55 and Pab 57.

Mouse and human tumor tissues are fixed in 10% neutral formalin. The tissues are then dehydrated, paraffin embedded and $10-20 \times 8$ -micron sections are cut for immunohistochemical staining.

Immunohistochemical analysis is performed with the automated Ventana Immunohistochemical Stainer according to the manufacturer's suggested protocol (Ventana, Tucson, Arizona). Sections are deparaffinated with xylene. The prepared sections are then treated with 1% hydrogen peroxide prepared in 30% aqueous methanol for 20 minutes at room temperature to quench endogenous peroxidase activity. The slides are then washed with PBS, blocked with 5% BSA and 5% goat serum in PBS, washed again and then incubated for 30 minutes at 37°C with the appropriate diluted (1:100) antibody. Horseradish peroxidase-labeled goat anti-rabbit IgG is used as a secondary antibody. For visualization, the sections are incubated with DAB/H₂O₂. The sections are finally incubated with a copper enhancer (Ventana) for 4 minutes, washed, counterstained with hematoxylin, and mounted in tolueneminus mounting medium. Photographic documentation is performed and images are stored for later review and analysis. The results are summarized in Table 9. The numbers refer to glass slides.

TABLE 9
Immunohistochemistry of tumor and normal tissues

(diff. = differentiated)

Human tissues:

5

10

15

·	Antibody	Magnification	Signal
Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 55	400x	+
2. Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 55	400x	+
3. Normal ovary (96-08ZO08) control tissue	Pab 55	400x	-
4. Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 57	400x	+
5. Ovary tumor (9 5 -02VO 16) high grade papillary carcinoma	Pab 57	400x	+
6. Ovary tumor (95-02VO16) high grade papillary carcinoma	Pab 57	400x	+
7. Normal ovary 96-08ZO08) control	Pab 57	400x	-
8. Colon cancer 95-14664) poorly diff. Adenocarcinoma	Pab 55	400x	+
9. Normal colon 9708VO08) control	Pab 55	400x	-
10. Colon cancer 95-14664) poorly diff. Adenocarcinoma	Pab 57	400x	+

WO 00/05375			PCT/US99/16676
11. Colon cancer 95-14664) poorly diff.	Pab 57	400x	+
Adenocarcinoma			
12. Normal colon 9708VO08) control	Pab 57	400x	-
13. Female breast cancer (97-IOV03a)	Pab 55	400x	. +
Invasive mammary carcinoma			
14. Male breast cancer (no code)	Pab 55	400x	+
mammary carcinoma			
15. Normal female breast 97-12VO20-3)	Pab 55	400x	•
control			
16. Female breast cancer 97-IOV03a)	Pab 57	400x	+
Invasive mammary carcinoma			
17. Male breast cancer (no code)	Pab 57	400x	+
mammary carcinoma			
18. Normal female breast (97-12VO20-3)	Pab 57	400x	•
control			
19. Lung cancer (97- 1 OV022-5) poorly	Pab 55	400x	+
diff. NOJ-small cell carcinoma	٠		
20. Normal lung (98-0 1 VO 11) control	Pab 55		•
21. Lung cancer (97-10VO22-5) poorly		400x	+
diff. NOJ-small cell carcinoma			
22. Lung cancer (97-10VO22-5) poorly	Pab 57	400x	+
diff. NOJ-small cell carcinoma			
23. Normal lung (98-0 1 VO 11) control	Pab 57		-

Mouse Tissues:

	Antibody	Magnification	Signal
24. Madison Lung Tumor (MLT) untreated with CM 101	Pab 55		+
25. MLT untreated with CM 101	Pab 55		+
26. Normal mouse lung	Pab 55		•
27. MLT untreated with CM 101	Pab 57		+
28. Normal mouse lung	Pab 57		•

cancer tissue section, but such staining is not apparent in cells of normal human ovary tissue (see FIG. 2A and 2B, respectively). Similar results are obtained with the Pab 57 antibody (see FIG. 3A and 3B). As shown in the above table and in FIGS. 2A-3B, antibodies raised to GBS toxin receptor fragments specifically bound to tumor tissues but not normal tissues, suggesting that GBS toxin receptor is expressed in tumor cells but not normal cells.

The Pab 55 antibody stains the cells lining a blood vessel in a human ovary

10 Example 5- Detection of GBS Toxin Receptor Expression in Mice Afflicted with Rheumatoid Arthritis

This example shows that GBS toxin receptor can be detected in cells from a mammalian model for rheumatoid arthritis (RA). Mice with collagen-induced arthritis were treated with CM101 or carrier. CM101 reversed the inflammatory

damage and inhibited pannus formation. Mouse #8 and #15, which were treated with CM101, and two control mice (not treated with CM101) were sacrificed for immunohistochemistry.

TABLE 10
Immunohistochemistry of Rheumatoid Arthritic Mice

Pab 55	+
Pab 55	+
Pab 57	+
	Pab 55 Pab 57 Pab 57 Pab 57 Pab 57 Pab 57

As shown above Pab55 and Pab57 specifically bound to pathologic neovasculature in the pannus, suggesting that GBS toxin receptor is expressed in mice afflicted with rheumatoid arthritis. No binding of CM101 was observed in the normal neovasculature in the growth plate of the joints of the arthritic mice.

EXAMPLE 6 – TARGETED DELIVERY OF A CHIMERIC COMPOUND TO TISSUES EXPRESSING GBS TOXIN RECEPTOR

This example shows the targeted delivery of a chimeric compound to tissues expressing GBS toxin. The chimeric compound is a CM101-biotin conjugate. Mice with Madison Lung Tumors (MLT) are infused intravenously (i.v.) with biotinylated CM 101.

CM101 has been reacted with hydrazinylated biotin to form the biotin
20 hydrazone at the reducing end of the polysaccharide CM101. Briefly, 25 micrograms
of lyophilized CM101 is dissolved in 250 µl labeling buffer at 100 mM sodium
acetate, 0.02% sodium azide. Aqueous meta-periodate (125 µl of 30 mM) is added
and the oxidation is allowed to proceed in the dark for 30 minutes at room
temperature. The reaction is terminated by adding 80 mM Na₂SO₃ to the solution.

The resultant aldehydes are reacted with 125 µl of 5 mM NHS-LC-Biotin (MW 556.58) for a 1 hour incubation at room temperature to form biotinylated CM101. Excess biotin is removed by dialysis against 1 liter of PBS at 4°C four times. The

5

10

product is purified by gel filtration on an Ultrahydrogel 1000 HPLC. lyophilized and stored at -70°C until use.

Tissues are recovered 5 min post infusion with CM101 and subjected to immunohistochemistry. Tumor and normal mouse tissue sections are analyzed for CM 101 binding by both mouse anti-CM101 mAb (7A3), followed by secondary mAb-HRP conjugate (referred to in FIG. 4B as MLT CM101-Biot.5' + McAb), or with avidin (which specifically binds biotin) conjugated with HRP (referred to in FIG. 4A as MLT CM101-Biot.5' + Strep.HRP).

FIGS. 4A-4C depict different sections taken from the same tumor and include a longitudinal view of the same blood vessel approximately in the center of the figures. The dark staining in FIG. 4A shows the localization of the biotin component in the cells lining the blood vessel. Similarly, FIG. 4B depicts the localization of the CM101 component in the cells lining the blood vessel. FIG. 4C is a negative control that was not exposed to CM101. The analysis clearly shows that 7A3 and avidin bind to the same blood vessels in tumor tissue. Thus, biotin has been delivered to the blood vessel of the tumor tissue by virtue of its physical association with a compound (CM101) that binds the GBS toxin receptor.

These studies show that chimeric compounds can be delivered to tissues undergoing pathologic and/or hypoxia-driven angiogenesis or neovascularization. As part of a chimeric compound, cytotoxic molecules can be directed to such tissues, e.g., tumor tissue. The cytotoxic molecule can be coupled directly to a molecule that binds GBS toxin receptor, e.g., GBS toxin. Alternatively, the molecule that binds GBS toxin receptor can be coupled to biotin and the cytotoxic molecule can be coupled to avidin.

5

10

15

EXAMPLE 7 – ENHANCED SENSITIVITY TO GBS-TOXIN-DEPENDENT CYTOTOXICITY OF CELLS EXPRESSING GBS TOXIN RECEPTOR

This example shows the enhanced sensitivity to GBS-toxin-dependent cytotoxicity of cells transfected with the GBS toxin receptor, relative to control cells. Without being bound to a particular theory, the inventors believe that complement binds GBS toxin bound to the GBS toxin receptor on a cell, thereby targeting the cell for killing by white blood cells (WBC).

Human bladder carcinoma cells (ECV cells), are stable transfected with the human GBS toxin receptor gene. The resultant cell line is ECV711. Cells stable transfected with vector alone as referred to as V23. ECV 711 and V23 are seeded in 96-well plates at 5,000 cells/well.

White blood cells are collected from healthy human donors as follows. Blood is collected by standard phlebotomy procedures into heparinized tubes (30 U/ml) and centrifuged at 2000 rpm for 20 min. The interface is carefully transferred to a new tube and washed twice by centrifugation with medium (RPMI-1640). Cells are resuspended in RPMI-1640 supplemented with 5% fetal bovine serum (FBS) and Interferon-gamma (IFN) at 100 U/ml, and incubated overnight in a 37°C, 5%CO₂ incubator. The cells are then resuspended in fresh medium with 5% FBS.

5,000 cells of the WBC preparation are added to each well containing the transfected cells. CM101 is added to a final concentration of $1\mu g/ml$ to the wells together with human serum from matching human donors. The cells are incubated 6 hours at 37 ° C.

Cytotoxicity is assayed by measuring lactate dehydrogenase (LDH) using the Promega's CytoTox 96 Non-Radioactive Assay kit (Nachlas et al. (1960) Anal. Biochem 1, 317; Korzeniewski et al. (1983) J. Immunol. Methods 64, 313; Decker et al. J. Immunol. Methods 115, 61; Brander et al. (1993) Eur. J. Immunology 23, 3217; Behl et al. (1994) Cell 77, 817; Lappalainen et al. (1994) Pharm. Research 11, 1127; Allen et al. (1994) Promega Notes 45, 7; Sinensky et al. (1995) Toxicol. Letters 75, 02; Moravec (1994) Promega Notes 45, 11). Percent cytotoxicity is calculated as recommended by the manufacturer's instructions. The results are shown in Table 11.

5

10

15

20

25

Table 11

Cytotoxicity	ECV 711	V 23
WBC, IFN, C3, -CM101	29.1%	27.5%
WBC, IFN, C3, +CM101	40.45%	22.46%

There is an increase in cytotoxicity of 39% when the ECV 711 cells are incubated with CM101, WBC and human serum (source of C3) compared to cells incubated without CM101. Control cells transfected with vector alone, V23, do not show a CM101 dependent increase in cytotoxicity.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

5

WHAT IS CLAIMED IS:

5

10

1. An isolated polynucleotide at least 10 bases in length comprising a nucleic acid sequence encoding, or complementary to a nucleic acid sequence encoding, a mammalian receptor for group B β-hemolytic Streptococcus toxin (GBS toxin receptor), or a polypeptide fragment thereof.

- 2. The polynucleotide of Claim 1, wherein the nucleic acid sequence comprises SEQ ID NO: 9.
- 3. The polynucleotide of Claim 1, wherein the nucleic acid sequence has 100% identity to a nucleic acid sequence selected from the group consisting of residues 61 to 1542 of SEQ ID NO: 1, residues 266 to 1870 of SEQ ID NO:7, and residues 87 to 1568 of SEQ ID NO: 3.
 - 4. The polynucleotide of Claim 1, wherein the polynucleotide is hybridizable under high stringency conditions to the nucleic acid sequence of SEQ ID NO: 7.
 - 5. A vector comprising the polynucleotide of Claim 1.
- 15 6. A host cell transformed with the vector of Claim 5.
 - 7. A process for producing a mammalian GBS toxin receptor or fragment thereof, comprising culturing the host cell of Claim 6 in a suitable culture medium.
 - 8. An isolated polypeptide comprising a mammalian GBS toxin receptor or fragment thereof.
- 9. The polypeptide of Claim 8, wherein the receptor has at least about 86% identity to the corresponding amino acid sequence of SEQ ID NO: 2.
 - 10. The polypeptide of Claim 8, wherein the receptor or fragment has 100% identity to the corresponding region of the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 8.
- 25 11. The polypeptide of Claim 8, wherein the polypeptide is encoded by a nucleic acid sequence hybridizable under high stringency conditions to a nucleic acid sequence selected from the group consisting of:

- a) nucleotides 61 to 1542 of SEQ ID NO: 1, and
- b) nucleotides 87 to 1568 of SEQ ID NO: 3.
- 12. An isolated polypeptide comprising an amino acid sequence that differs from an amino acid sequence selected from the group consisting of SEQ ID NO:2,
- SEQ ID NO:4 and SEQ ID NO:8 at no more than about 20% of the amino acid residues.
 - 13. The isolated polypeptide of Claim 12, wherein the amino acid sequence of said isolated polypeptide differs from the amino acid sequence selected from said group by one amino acid residue.
- 10 14. The isolated polypeptide of Claim 12, wherein the different amino acid residues are conservative substitutions of the corresponding residues of the amino acid sequence selected from said group.
 - 15. An isolated polypeptide comprising an amino acid sequence of the formula:

15

20

25

AA1-AAn-AAm

wherein:

AA1 is absent or is M;

AAn is a contiguous chain of 0 to 100 amino acids, preferably of 0 or 41 amino acids, even more preferably of residues 2-42 of SEQ ID NO:8; and

AAm is a contiguous chain of 494 amino acids comprising AA43 through AA536, wherein:

(1) each of AA43, AA47, AA51, AA52, AA57, AA58, AA65, AA66, AA72, AA85, AA87, AA93, AA94, AA96, AA115, AA116, AA122, AA123, AA125, AA134, AA143, AA173, AA174, AA178, AA185, AA186, AA189, AA190, AA196, AA200, AA204, AA206, AA207, AA220, AA253, AA260, AA276, AA277, AA280, AA283, AA287, AA294, AA295, AA298, AA300, AA301, AA312, AA324, AA326, AA360, AA365, AA373, AA374, AA379, AA396, AA403, AA407, AA418, AA480, AA483, AA486, AA491, AA494, AA502, AA528, AA529, AA532 and AA536 is an amino acid residue corresponding to:

30 (a) residue 43, 47, 51, 52, 57, 58, 65, 66, 72, 85, 87, 93, 94, 96, 115, 116, 122, 123, 125, 134, 143, 173, 174, 178, 185, 186, 189, 190, 196, 200, 204, 206, 207, 220, 253, 260, 276, 277, 280, 283, 287, 294, 295, 298, 300, 301, 312, 324, 326, 360, 365, 373, 374, 379, 396, 403, 407, 418, 480, 483, 486, 491, 494, 502, 528, 529, 532 and 536, respectively, of SEQ ID NO:8;

35 (b) residue 2, 6, 10, 11, 16, 17, 24, 25, 31, 44, 46, 52, 53, 55, 74, 75, 81, 82, 84, 93, 102, 132, 133, 137, 144, 145, 148, 149, 155, 159, 163, 165, 166, 179, 212, 219, 235, 236, 239, 242, 246, 253, 254, 257, 259, 260, 271, 283,

285, 319, 324, 332, 333, 338, 355, 362, 366, 377, 439, 442, 445, 450, 453, 461, 487, 488, 491 and 495, respectively of SEQ ID NO:4; or

(c) a conservative substitution thereof;

- (2) each of AA44-AA46, AA48-AA50, AA53-AA56, AA59-AA64, AA67-AA71, AA73-AA84, AA86, AA88-AA92, AA95, AA97-AA114, AA117-AA121, AA124, AA126-AA133, AA135-AA142, AA144-AA172, AA175-AA177, AA179-AA184, AA187-AA188, AA191-AA195, AA197-AA199, AA201-AA203, AA205, AA208-AA219, AA221-AA252, AA254-AA259, AA261-AA275, AA278-AA279, AA281-AA282, AA284-AA286, AA288-AA293, AA296-AA297, AA299, AA302-AA311, AA313-AA323, AA325, AA327-AA359, AA361-AA364, AA366-
- 10 AA302-AA311, AA313-AA323, AA325, AA327-AA359, AA361-AA364, AA366-AA372, AA375-AA378, AA380-AA395, AA397-AA402, AA404-AA406, AA408-AA417, AA419-AA478, AA481-AA482, AA484-AA485, AA487-AA490, AA492-AA493, AA495-AA501, AA503-AA527, AA530-AA531 and AA533-AA535 is

 (a) residue 44-46, 48-50, 53-56, 59-64, 67-71, 73-84,
- 15 86, 88-92, 95, 97-114, 117-121, 124, 126-133, 135-142, 144-172, 175-177, 179-184, 187-188, 191-195, 197-199, 201-203, 205, 208-219, 221-252, 254-259, 261-275, 278-279, 281-282, 284-286, 288-293, 296-297, 299, 302-311, 313-323, 325, 327-359, 361-364, 366-372, 375-378, 380-395, 397-402, 404-406, 408-417, 419-478, 481-482, 484-485, 487-490, 492-493, 495-501, 503-527, 530-531 and 533-535, respectively, of SEQ ID NO:8; or
 - (b) a conservative substitutions thereof; and (3) one or more of AA315 through AA367 are optionally absent.
 - 16. An antibody that recognizes a mammalian GBS toxin receptor or fragment thereof.
- 25 17. An isolated complex comprising a GBS toxin bound to a mammalian GBS toxin receptor or fragment thereof.
 - 18. A method of forming a complex comprising:

contacting a GBS toxin with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that permit specific binding of the GBS toxin to the polypeptide, and allowing the complex to form.

19. A method for purifying a compound that binds a GBS toxin receptor, which method comprises:

providing a polypeptide comprising a mammalian GBS toxin receptor or fragment thereof that binds GBS toxin;

contacting said polypeptide with a sample comprising the compound under conditions that allow specific binding of the compound to the polypeptide; and

separating the bound compound from the remainder of the sample.

20. A method of determining the presence or absence of GBS toxin in a sample, which method comprises:

contacting the sample with a polypeptide comprising a mammalian

GBS toxin receptor, or fragment thereof that can bind GBS toxin, under conditions that permit specific binding of the GBS toxin to the polypeptide,

and determining whether specific binding has occurred.

- 21. A method for diagnosing early onset disease in a neonate comprising performing the method of Claim 20, wherein the sample is obtained from the neonate and wherein presence of the GBS toxin is indicative of early onset disease.
 - 22. A method for detecting pathologic vasculature in a mammalian tissue, which method comprises detecting the presence of a GBS toxin receptor.
 - 23. A method for identifying a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor, comprising:
- 15 combining a test compound with a polypeptide comprising a mammalian GBS toxin receptor, or fragment thereof that can bind GBS toxin, in a reaction mixture containing GBS toxin and under conditions that permit specific binding of the GBS toxin to the receptor or fragment, and
- determining the amount of inhibition by the compound of the binding of the GBS toxin to the polypeptide.
 - 24. An inhibitor of binding of a GBS toxin to a mammalian GBS toxin receptor.
 - 25. A method for identifying a compound which specifically binds a mammalian GBS toxin receptor, comprising:
- combining a test compound with a polypeptide comprising a mammalian GBS toxin receptor or fragment thereof that can bind GBS toxin, under conditions that allow specific binding to occur, and

detecting a complex formed between said test compound and said

polypeptide.

26. A method for determining cytotoxicity of a test chimeric compound, which method comprises:

exposing a cell expressing, on the cell surface, a mammalian GBS

toxin receptor, or fragment thereof that binds GBS toxin, to a test chimeric compound comprising a cytotoxic agent coupled to said GBS toxin; and detecting signs of cytotoxicity.

- 27. A chimeric compound comprising a cytotoxic agent covalently linked to a molecule that specifically binds a mammalian GBS toxin receptor.
- 10 28. A method for identifying an inhibitor of GBS toxin receptor, which method comprises:

incubating test cells in the presence and absence of a test compound and under conditions in which the cells incubated in the absence of the test compound can proliferate or migrate, wherein the test cells express GBS toxin receptor or a fragment thereof having GBS toxin receptor activity; and

comparing the proliferation or migration of the test cells incubated in the presence of the test compound to the proliferation or migration of the test cells incubated in the absence of the test compound, wherein less proliferation or migration in the presence of the test compound is indicative of the test compound being an inhibitor of the GBS toxin receptor.

29. A method for identifying an inhibitor of endothelial cell proliferation or migration, which method comprises:

incubating test endothelial cells in the presence and absence of a test compound and under conditions in which the cells incubated in the absence of the test compound can proliferate or migrate, wherein the test cells express GBS toxin receptor or a fragment thereof having GBS toxin receptor activity; and

comparing the proliferation or migration of the test cells incubated in the presence of the test compound to the proliferation or migration of the test cells incubated in the absence of the test compound, wherein less proliferation or migration

15

20

in the presence of the test compound is indicative of the test compound being an inhibitor of the endothelial cell proliferation or migration.

30. A method for identifying a therapeutic compound for the treatment or prevention of a medical condition characterized by pathologic angiogenesis or neovascularization, which method comprises:

incubating test cells in the presence and absence of a test compound, wherein the test cells express GBS toxin receptor or a fragment thereof having GBS toxin receptor activity;

comparing the proliferation or migration of the test cells incubated in
the presence of the test compound to the proliferation or migration of the test cells
incubated in the absence of the test compound, wherein less proliferation or migration
in the presence of the test compound is indicative of the test compound being a
candidate therapeutic compound for the treatment or prevention of the medical
condition.

- 15 31. The method of Claim 30, wherein the medical condition is a cancerous tumor.
 - 32. The method of Claim 30, wherein the medical condition is a reperfusion injury.
- 33. The method of Claim 30, wherein the medical condition is scarring during wound healing.
 - 34. The method of Claim 30, wherein the medical condition is keloids.
 - 35. The method of Claim 30, wherein the medical condition is a chronic inflammatory disease.
 - 36. The method of Claim 30, wherein the medical condition is neural injury.
- 25 37. A method for identifying a compound which inhibits binding of a GBS toxin to a mammalian GBS toxin receptor, comprising:
 - (a) simulating and selecting the most probable conformations of a mammalian GBS toxin receptor,

(b) designing a chemically modified analog that substantially mimics the energetically most probable three-dimensional structure of the polypeptide,

- (c) chemically synthesizing the analog, and
- (d) evaluating the bioactivity of the analog.
- 5 38. A method for identifying a compound which binds to a mammalian GBS toxin receptor, comprising:
 - (a) simulating and selecting the most probable conformations of a mammalian GBS toxin receptor,
 - (b) deducing the most probable binding domains of the polypeptide,
- 10 (c) designing a compound that would form the energetically most probable complexes with the polypeptide,
 - (d) chemically synthesizing the compound, and
 - (e) evaluating the bioactivity of the compound.
- 39. A method for the prevention or treatment of neonatal onset disease in a human neonate, comprising administering an inhibitor of binding of GBS toxin to a human GBS toxin receptor.
 - 40. A method for inhibiting pathologic or hypoxia-driven endothelial cell proliferation or migration in a mammalian tissue, which method comprises specifically binding a molecule to a GBS toxin receptor present on the surface of at least one cell in the tissue, the molecule being selected from the group consisting of:
 - a compound that can evoke an inflammatory response when bound to a GBS toxin receptor in a mammal;
 - a chimeric compound comprising a cytotoxic compound coupled to a compound that specifically binds the GBS toxin receptor;
 - an inhibitor of GBS toxin receptor phosphorylation; and an inhibitor of GBS toxin receptor activity.
 - 41. A pharmaceutical composition comprising a pharmaceutically effective amount of a molecule selected from the group consisting of:
 - a GBS toxin receptor or fragment thereof;

20

25

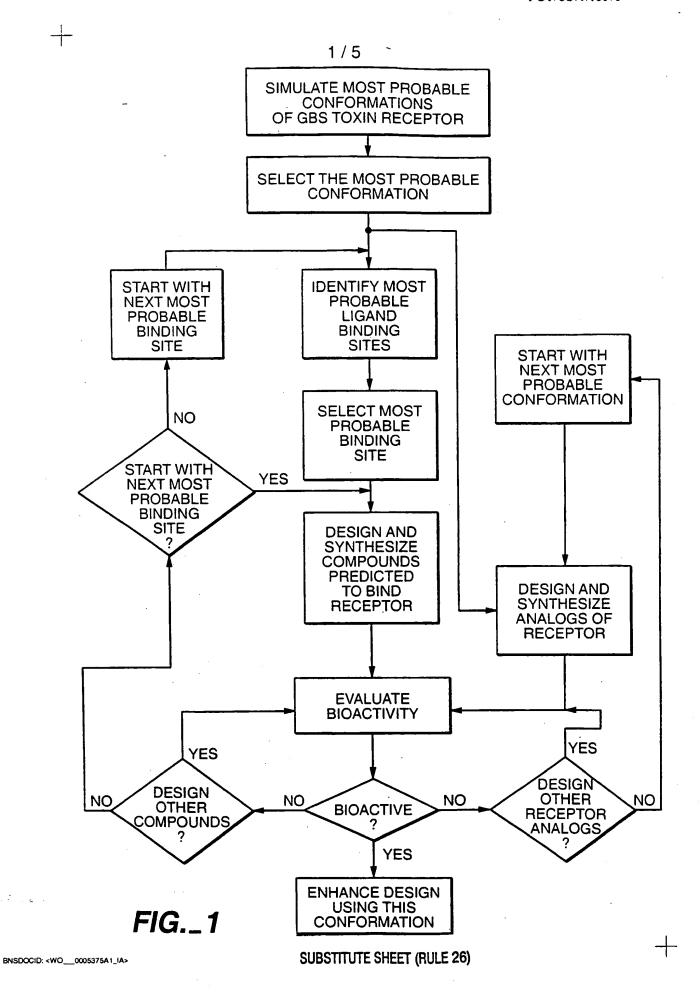
an inhibitor of a GBS toxin receptor; and

a chimeric compound comprising a cytotoxic agent coupled to a compound that binds GBS toxin receptor, and a pharmaceutically acceptable carrier.

5

20

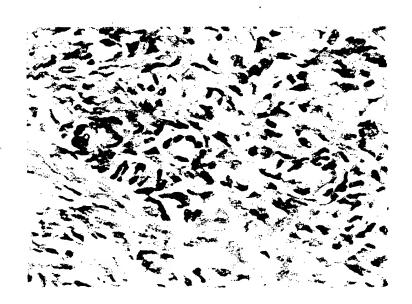
- 42. A kit comprising a component selected from the group consisting of:
 - a GBS toxin receptor or fragment;
- a reagent for detecting the presence of a GBS toxin receptor or fragment; and
- a reagent for detecting the presence of polynucleotide encoding the GBS toxin receptor or fragment.
 - 43. A molecule for use in a method of treatment of the human or animal body, said molecule being selected from the group consisting of:
- a GBS toxin receptor or fragment thereof for use in a method of treatment of the human or animal body, said molecule being selected from the group consisting of:
 - a GBS toxin receptor or fragment thereof; an inhibitor of binding of GBS toxin to a GBS toxin receptor; an inhibitor of a GBS toxin receptor; and
 - a chimeric compound comprising a cytototoxic agent coupled to a compound that binds GBS toxin receptor.
- 44. Use of an inhibitor of a GBS toxin receptor, or of an inhibitor of binding of GBS toxin to a GBS toxin receptor, for the manufacture of a medicament for the treatment of a medical condition characterized by pathologic or hypoxia-driven
 angiogenesis or neovascularization.





HUMAN OVARY CANCER + Pab 55

FIG._2A



NORMAL HUMAN OVARY + Pab 55

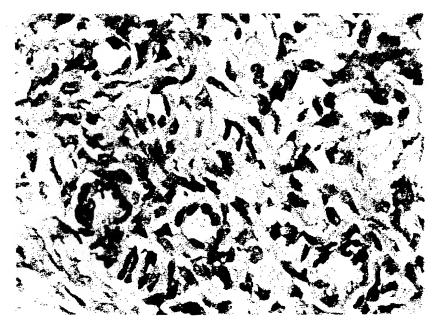
FIG._2B

SUBSTITUTE SHEET (RULE 26)



HUMAN OVARY CANCER + Pab 57

FIG._3A



NORMAL HUMAN OVARY + Pab 57

FIG._3B
SUBSTITUTE SHEET (RULE 26)

4/5



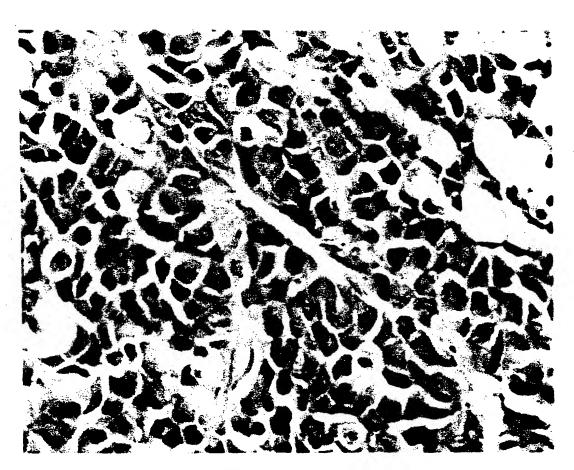
MLT CM101 - Biot.5'+ Strep.HRP

FIG._4A



MLT CM101 - Biot.5' + mAb

FIG._4BSUBSTITUTE SHEET (RULE 26)



MLT - PBS 5' + Streptavidin - HRP

FIG._4C

SEQUENCE LISTING

	erqvist, Ca	rl				
_ ru, (Changlin					
<120> GBS '	Toxin Recep	tor				
<130> CARB	-008/01WO					
<140>						
<141>						
<150> 60-0	93,843					
<151> 1998·	-07-22					
<160> 12						
<170> Pater	ntIn Ver. 2	.0				
<210> 1						
<211> 2602						
<212> DNA						
<213> Homo	sapiens				•	
<220>						
<221> CDS						
<222> (58).	(1542)					
<400> 1						
tcgggccggc	gctcccttct	ctgccaggt	g gcgagtad	cac ctgctcad	gt aggegte	57
					gag gag agc	105
Met Arg Ser		rg Asp Leu	Ala Arg A	Asn Asp Gly	Glu Glu Ser	
1	5		10		15 .	
					gaa gcc gct	153
Thr Asp Arg				Pro Arg Ala	Glu Ala Ala	
	20		25		30	
cca gtg tgc	tgc tct go	ct cgt tac	aac tta g	ca att ttg	gcc ttt ttt	201
Pro Val Cys	Cys Ser Al	la Arg Tyr	Asn Leu A	la Ile Leu	Ala Phe Phe	
35	i	40		45		
ggt ttc ttc	att gtg ta	it gca tta	cgt gtg a	at ctg agt	gtt gcg tta	249
Gly Phe Phe	: Ile Val Ty	r Ala Leu	Arg Val A	sn Leu Ser	Val Ala Leu	
50		55		60		
gtg gat atg	gta gat to	a aat aca	act tta g	aa gat aat	aga act tcc	297

Val 65	Asp	Met	Val	Asp	Ser 70	Asn	Thr	Thr	Leu	Glu 75		Asn	Arg	Thr	Ser 80	
_															caa Gln	345
_															ctc Leu	393
							atc Ile 120									441
	_						aaa Lys								ctt Leu	489
		_	-				ttc Phe								gga Gly 160	537
-							aga Arg									585
_							gcc Ala									633
	_	_	_				agc Ser 200									681
							ctt Leu									729
							ttt Phe									777
		_					gtt Val									825
aga	att	tcc	cat	tat	gaa	aag	gaa	tac	att	ctt	tca	tca	tta	aga	aat	873

Arg	Ile	Ser	His 260	Tyr	Glu	Lys	Glu	Tyr 265	Ile	Leu	Ser	Ser	Leu 270	Arg	Asr.	
cag	ctt	tct	tca	cag	aag	tca	gtg	ccg	tgg	gta	ccc	att	tta	aaa	tcc	921
Gln	Leū	Ser	Ser	Gln	Lys	Ser	Val	Pro	Trp	Val	Pro	Ile	Leu	Lys	Ser	
		275					280					285				
ctg	cça	ctt	tgg	gct	atc	gta	gtt	gca	cac	ttt	tct	tac	aac	tgg	act	969
Leu	Pro	Leu	Trp	Ala	Ile	Val	Val	Ala	His	Phe	Ser	Tyr	Asn	Trp	Thr	
	290					295					300					
				_		tta	_					_				1017
	Tyr	Thr	Leu	Leu		Leu	Leu	Pro	Thr	-	Met	Lys	Glu	Ile		
305					310					315	•				320	1000
			-			aat						_				1065
Arg	Pne	Asn	vaı		GIU	Asn	GIA	Pne		ser	ser	ren	Pro	-	ren	
				325				•	330					335		
ggc	tct	tgg	tta	tgt	atg	atc	ctg	tct	ggt	caa	gct	gct	gac	aat	tta	1113
Gly	Ser	Trp	Leu	Cys	Met	Ile	Leu	Ser	Gly	Gln	Ala	Ala	Asp	Asn	Leu	
			340					345					350			
	_					tca										1161
Arg	Ala	Lys 355	Trp	Asn	Phe	Ser	Thr 360	Leu	Cys	Val	Arg	Arg 365	Ile	Phe	Ser	
ctt	ata	gga	atg	att	gga	cct	gca	gta	ttc	ctg	gta	gct	gct	ggc	ttc	1209
Leu	Ile	Gly	Met	Ile	Gly	Pro	Ala	Val	Phe	Leu	Val	Ala	Ala	Gly	Phe	
	370					375					380					
		_	_			ttg	-	_	-				•			1257
Ile	Gly	Cys	Asp	Tyr	Ser	Leu	Ala	Val	Ala		Leu	Thr	Ile	Ser		
385					390					395					400	
aca	ctg	gga	ggc	ttt	tgc	tct	tct	gga	ttt	agc	atc	aac	cat	ctg	gat	1305
Thr	Leu	Gly	Gly	Phe	Cys	Ser	Ser	Gly	Phe	Ser	Ile	Asn	His	Leu	Asp	
				405					410					415		
						ggt										1353
Ile	Ala	Pro	Ser	Tyr	Ala	Gly	Ile	Leu	Leu	Gly	Ile	Thr	Asn	Thr	Phe	
			420					425					430			
						gtt										1401
Ala	Thr	Ile	Pro	Gly	Met	Val	Gly	Pro	Val	Ile	Ala	Lys	Ser	Leu	Thr	
		435					440					445				
cct	gat	aac	act	gtt	gga	gaa	tgg	caa	acc	gtg	ttc	tat	att	gct	gct	1449

Pro Asp Asn Thr Val Gly Glu Trp Gln Thr Val Phe Tyr Ile Ala Ala 450 455 460

gct att aat gtt ttt ggt gcc att ttc ttt aca cta ttc gcc aaa ggt 1495
Ala Ile Asn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly
465 470 475 480

gaa gta caa aac tgg gct ctc aat gat cac cat gga cac aga cac
1542
Glu Val Gln Asn Trp Ala Leu Asn Asp His His Gly His Arg His
485
490
495

tgaaggaacc aataaataat cctgcctcta ttaatgtatt tttatttatc atgtaacctc 1602 aaaqtgcctt ctgtattgtg taagcattct atgtcttttt ttaattgtac ttgtattaga 1662 tttttaaggc ctataatcat gaaatatcac tagttgccag aataataaaa tgaactgtgt 1722 ttaattatga ataatatgta agctaggact tctactttag gttcacatac ctgcctgcta 1782 gtcgggcaac atgaagtagg acagttctgt tgatttttta gggccatact aaagggaatg 1842 agctgaaaca gacctcctga tacctttgct taattaaact agatgataat tctcaggtac 1902 tgataaacac ctgttgttgt tcactttcct cataaaaatt gtcagctctc tctgacactt 1962 agacctcaaa ctttagcatc tctgtggagc tgccatccac tgtataattt cgcctqqcaa 2022 ctggactgag gggagtgtgc ccaggcagct gccaagcact ccctccctgg cttcagggtc 2082 agagtgccca gcgtttatca gaggcagcat ccaagcccag agccagtgtc gactcttcgg 2142 ctggtgcctt tcctctgagg ggctatcaat gtgtagataa agccctgagt aggcaagagc 2202 agtgagatec actgetatgg tettgataca tecteaaact tteeetteec ageacagagg 2262 aatattggct ggcatgcaac ctgcaaaaga aaaatgcgaa gcggccgggc acggtggctc 2322 atgeetgtaa teecageaet ttgggggget gaggtgggeg aateatgaga teaggagtte 2382 gagaccagee tggccageat ggtgaaacce catetetact aaaaatacaa aaaattaget 2442 gggcgtggtg acgggcgcct gtaatcccag atactcagga ggctgaggta ggagaatcac 2502 ttgaacctgg gaggtggaag ttgcagtgaa ccaagatcac gccactgcac tccagcctgg 2562 2602

<210> 2

<211> 495

<212> PRT

<213> Homo sapiens

<400> 2

Met Arg Ser Pro Val Arg Asp Leu Ala Arg Asn Asp Gly Glu Glu Ser

1 5 10 15

Thr Asp Arg Thr Pro Leu Leu Pro Gly Ala Pro Arg Ala Glu Ala Ala 20 25 30

Pro Val Cys Cys Ser Ala Arg Tyr Asn Leu Ala Ile Leu Ala Phe Phe 35 40 45

Gly Phe Phe Ile Val Tyr Ala Leu Arg Val Asn Leu Ser Val Ala Leu 50 55 60

Val Asp Met Val Asp Ser Asn Thr Thr Leu Glu Asp Asn Arg Thr Ser 65 70 75 80

Lys Ala Cys Pro Glu His Ser Ala Pro Ile Lys Val His His Asn Gln 85 90 95

Thr Gly Lys Lys Tyr Gln Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu
100 105 110

Gly Ser Phe Phe Tyr Gly Tyr Ile Ile Thr Gln Ile Pro Gly Gly Tyr
115 120 125

Val Ala Ser Lys Ile Gly Gly Lys Met Leu Leu Gly Phe Gly Ile Leu 130 135 140

Gly Thr Ala Val Leu Thr Leu Phe Thr Pro Ile Ala Ala Asp Leu Gly
145 150 155 160

Val Gly Pro Leu Ile Val Leu Arg Ala Leu Glu Gly Leu Gly Glu Gly 165 170 175

Val Thr Phe Pro Ala Met His Ala Met Trp Ser Ser Trp Ala Pro Pro 180 185 190

Leu Glu Arg Ser Lys Leu Leu Ser Ile Ser Tyr Ala Gly Ala Gln Leu 195 200 205

Gly Thr Val Ile Ser Leu Pro Leu Ser Gly Ile Ile Cys Tyr Tyr Met 210 215 220

Asn Trp Thr Tyr Val Phe Tyr Phe Phe Gly Thr Ile Gly Ile Phe Trp 225 230 235 240

- Phe Leu Leu Trp Ile Trp Leu Val Ser Asp Thr Pro Gln Lys His Lys
 245 250 255
- Arg Ile Ser His Tyr Glu Lys Glu Tyr Ile Leu Ser Ser Leu Arg Asn 260 265 270
- Gln Leu Ser Ser Gln Lys Ser Val Pro Trp Val Pro Ile Leu Lys Ser 275 280 285
- Leu Pro Leu Trp Ala Ile Val Val Ala His Phe Ser Tyr Asn Trp Thr 290 295 300
- Phe Tyr Thr Leu Leu Thr Leu Leu Pro Thr Tyr Met Lys Glu Ile Leu 305 310 315 320
- Arg Phe Asn Val Gln Glu Asn Gly Phe Leu Ser Ser Leu Pro Tyr Leu 325 330 335
- Gly Ser Trp Leu Cys Met Ile Leu Ser Gly Gln Ala Ala Asp Asn Leu 340 345 350
- Arg Ala Lys Trp Asn Phe Ser Thr Leu Cys Val Arg Arg Ile Phe Ser 355 360 365
- Leu Ile Gly Met Ile Gly Pro Ala Val Phe Leu Val Ala Ala Gly Phe 370 375 380
- Ile Gly Cys Asp Tyr Ser Leu Ala Val Ala Phe Leu Thr Ile Ser Thr 385 390 395 400
- Thr Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Asp 405 410 415
- Ile Ala Pro Ser Tyr Ala Gly Ile Leu Leu Gly Ile Thr Asn Thr Phe
 420 425 430
- Ala Thr Ile Pro Gly Met Val Gly Pro Val Ile Ala Lys Ser Leu Thr 435 440 445
- Pro Asp Asn Thr Val Gly Glu Trp Gln Thr Val Phe Tyr Ile Ala Ala 450 455 460
- Ala Ile Asn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly
 465 470 475 480

Glu Val Gln Asn Trp Ala Leu Asn Asp His His Gly His Arg His
485
490
495

<210>-3

<211> 2844

<212> DNA

<213> Ovis sp.

<220>

<221> CDS

<222> (84)..(1568)

<400> 3

cccgggggcg gggggcttcg gcggtcccgc tggagctctc ttttccgcgg agcaggtttg 60

cgccgtagct ccctgaaggc atc atg aag tcc ccg gtt tcg gac tta gcc ccg 113

Met Lys Ser Pro Val Ser Asp Leu Ala Pro

1 5 10

age gac gge gag gge teg gac ege aca eeg ete etg eag ege gee 161 Ser Asp Gly Glu Glu Gly Ser Asp Arg Thr Pro Leu Leu Gln Arg Ala 15 20 25

ccg cgg gcg gaa ccc gct cca gta tgc tgc tct gct cgt tac aac cta 209
Pro Arg Ala Glu Pro Ala Pro Val Cys Cys Ser Ala Arg Tyr Asn Leu
30 35 40

gca ttt ttg tcc ttt ttt ggt ttc ttc gtt ctc tat tca tta cgg gtg 257
Ala Phe Leu Ser Phe Phe Gly Phe Phe Val Leu Tyr Ser Leu Arg Val
45 50 55

aat ctg agc gtt gca cta gtg gac atg gtg gat tca aac aca act gcc 305
Asn Leu Ser Val Ala Leu Val Asp Met Val Asp Ser Asn Thr Thr Ala
60 65 70

aaa gat aat aga acg tcc tac gag tgt gca gag cat tct gct ccc ata 353
Lys Asp Asn Arg Thr Ser Tyr Glu Cys Ala Glu His Ser Ala Pro Ile
75 80 85 90

aaa gtt ctt cac aac caa acg ggt aaa aag tac cgg tgg gat gca gaa 401 Lys Val Leu His Asn Gln Thr Gly Lys Lys Tyr Arg Trp Asp Ala Glu 95 · 100 105

act cas ggs tgg att ctc ggs tct ttt ttc tat ggc tac atc atc aca 449
Thr Gln Gly Trp Ile Leu Gly Ser Phe Phe Tyr Gly Tyr Ile Ile Thr
110 115 120

caa	att	cct	gga	gga	tat	gtt	gcc	agc	aga	agt	999	_ 3 33	aag	ctg	ttg	497
Gln	Ile	Pro	Gly	Gly	Tyr	Val	Ala	Ser	Arg	Ser	Gly	Gly	Lys	Leu	Leu	
		125					130					135	,			
cta	gga	ttc	aaa	atc	ttt	act	aca	act	ato	tto	acc	cta	tto	act	ccc	545
						-		_			Thr					
пец	140	F 11.C	GI	***	FILE		1111	AIG	110		150		FILE	1111	PIO	
	140					145					130					
	_					-					gca			_		593
Leu	Ala	Ala	Asp	Phe		Val	Gly	Ala	Leu		Ala	Leu	Arg	Ala	Leu	
155					160					165					170	
gaa	999	cta	gga	gag	ggt	gtc	aca	tat	cca	gcc	atg	cat	gcc	atg	tgg	641
Glu	Gly	Leu	Gly	Glu	Gly	Val	Thr	Tyr	Pro	Ala	Met	His	Ala	Met	Trp	
				175					180					185	_	
tct	tica	taa	act	ccc	cct	ctt	gaa	aσa	agc	aaσ	ctt	cta	agt	att	tica	689
			_				-	_	_	_	Leu	_	. –			005
501	561	115	190			200		195		-,,			200		Jei	
			130					193					200			
	_		_								ctt -					737
Tyr	Ala	_	Ala	Gln	Leu	GIA		Val	Val	Ser	Leu		Leu	Ser	Gly	
		205					210					215				
				•												
gta	att	tgc	tac	tat	atg	aat	tgg	act	tat	gtc	ttc	tat	ttc	ttt	ggc	785
Val	Ile	Cys	Tyr	Tyr	Met	Asn	Trp	Thr	Tyr	Val	Phe	Tyr	Phe	Phe	Gly	
	220					225					230					
att	gtt	gga	atc	atc	tgg	ttt	att	tta	tgg	atc	tgc	tta	gtt	agt	gat	833
Ile	Val	Gly	Ile	Ile	Trp	Phe	Ile	Leu	Trp	Ile	Cys	Leu	Val	Ser	Asp	
235		_			240				_	245	_				250	
aca	cca	gaa	act	cac	аас	aca	atc	act	ccc	tat	gaa	aac	gag	tat	att	881
		_			_						Glu	_				001
1111	-10	GIG	****	255	Lys	* ***	110		260	-1-	914	_,_	014	265	110	
				233					200					203		
																000
						_				_	aag			-		929
Leu	Ser	Ser		Lys	Asn	Gin	Leu		Ser	GIN	Lys	Ser		Pro	Trp	
			270					275					280			
ata	cct	atg	ctg	aaa	tca	ctg	cca	ctt	tgg	gct	att	gtc	gtt	gca	cat	977
Ile	Pro	Met	Leu	Lys	Ser	Leu	Pro	Leu	\mathtt{Trp}	Ala	Ile	Val	Val	Ala	His	
		285					290					295				
ttt	tct	tac	aac	tga	act	ttt	tat	act	ttg	ttg	acc	tta	ttg	cct	act	1025
											Thr					
	300	-1-		F		305	-1-				310			-	-	

														tta Leu 330	1073
				Leu										ggt Gly	1121
								tgg Trp					_		1169
								atg Met							1217
_	-							gat Asp			_	_	-	-	1265
								ggc							1313
_								tcg Ser 420						_	1361
								cct Pro		-					1409
	_	_	_					act Thr							1457
_		_						gta Val							1505
			_		_			aac Asn		-		_	_		1553
		cac His	_	tgaa	ggaa	icc a	ataa	ataa	it co	tgto	tcta	tta	atgt	atc	1608

tttgtttatc atgtaaccta aaagtgcctt tgatatttta atgtgtaagc aatctatata 1668 caagataaaa ttgtactaga aaaattgtgt tagatttgta aggcttgtaa tcatgaaatg 1728 teactagttg ceatataage aaaattaget atttttaatt attattaace egtttgetgg 1788 aacttacaat tcagggtcac atatotggct gcaagtcagg caacccacaa taggggagtt 1848 ctatttattt ataagaccat acctaaagag atgagctgaa atagaccctt ctataccttt 1908 qcttaattaa ggtggataat aattctcagg tcttgttaaa catctgtttt tgtacacctt 1968 cctcaaaaaa ttatttgtca tcagcaatcc ctgacatgta ggtctcaaac tttagcctct 2028 ccacggaget ggcagecact gtateattea geetggcaae tteactgagg gaageatgee 2088 caggragety ccacatytee cetetetyge tteagggaca gtgcccagea ettaggcage 2148 atccaagacc agggtcagcg ccaaggcttt ggacggtatt cttcccctgg ggctgttaat 2208 qtqtqqatqa aqccctqagc caacagggac agcgcgatcc acagtcatgg tttccatgca 2268 centetecet tecettecca geacactgga gtattgeetg geatgtaace tgcaaaagaa 2328 aqtqtqatqc ctaattagcc acatataaca tcatccttga tgatcctacc ttcacatgga 2388 tcagagtata aatcttcaag tcctgtgttc taggagctac accagaataa ttaaaatata 2448 aaaaqaaaca aaacattttt tetgtetgae acetaagtgt etggttgeag tteaaggtta 2508 aagtgacttc tacttcacat aacctgcaac cggtggtgta atcatcttta gtgttggttt 2568 cttaaatctt atttttccag ttittcctgg accatcttcc agtggttttg agcatgcttt 2628 gagggcattt atgtgattta gaacttgatt aatgtttcac tgtgtatgtt caacactacc 2688 tgtaatattt taactaaagc tatttaatgt aatatgatgt gtatacattc tgtaaattaa 2748 tttttaaatc tgtaaatagc tttaagttgc tatggtgata tttcttttac aaatcaaaat 2808 2844 aaatcttttt ggaatgataa aaaaaaaaaa aaaaaa

<210> 4

<211> 495

<212> PRT

<213> Ovis sp.

_	4	Λ	^	>	
•	4	u	u	>	- 4

Met	Lys	Ser	Pro	Val	Ser	Asp	Leu	Ala	Pro	Ser	Asp	Gly	Glu	Glu	Gly
1				5					10					15	

- Ser Asp Arg Thr Pro Leu Leu Gln Arg Ala Pro Arg Ala Glu Pro Ala
 20 25 30
- Pro Val Cys Cys Ser Ala Arg Tyr Asn Leu Ala Phe Leu Ser Phe Phe 35 40 45
- Gly Phe Phe Val Leu Tyr Ser Leu Arg Val Asn Leu Ser Val Ala Leu 50 55 60
- Val Asp Met Val Asp Ser Asn Thr Thr Ala Lys Asp Asn Arg Thr Ser 65 70 75 80
- Tyr Glu Cys Ala Glu His Ser Ala Pro Ile Lys Val Leu His Asn Gln 85 90 95
- Thr Gly Lys Lys Tyr Arg Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu 100 105 110
- Gly Ser Phe Phe Tyr Gly Tyr Ile Ile Thr Gln Ile Pro Gly Gly Tyr
 115 120 125
- Val Ala Ser Arg Ser Gly Gly Lys Leu Leu Leu Gly Phe Gly Ile Phe 130 135 140
- Ala Thr Ala Ile Phe Thr Leu Phe Thr Pro Leu Ala Ala Asp Phe Gly
 145 150 155 160
- Val Gly Ala Leu Val Ala Leu Arg Ala Leu Glu Gly Leu Gly Glu Gly
 165 170 175
- Val Thr Tyr Pro Ala Met His Ala Met Trp Ser Ser Trp Ala Pro Pro 180 185 190
- Leu Glu Arg Ser Lys Leu Leu Ser Ile Ser Tyr Ala Gly Ala Gln Leu 195 200 205
- Gly Thr Val Val Ser Leu Pro Leu Ser Gly Val Ile Cys Tyr Tyr Met 210 215 220
- Asn Trp Thr Tyr Val Phe Tyr Phe Phe Gly Ile Val Gly Ile Ile Trp 225 230 235 240
- Phe Ile Leu Trp Ile Cys Leu Val Ser Asp Thr Pro Glu Thr His Lys
 245 250 255

Thr Ile Thr Pro Tyr Glu Lys Glu Tyr Ile Leu Ser Ser Leu Lys Asn 260 265 270

Gln Leu Ser Ser Gln Lys Ser Val Pro Trp Ile Pro Met Leu Lys Ser 275 280 285

Leu Pro Leu Trp Ala Ile Val Val Ala His Phe Ser Tyr Asn Trp Thr 290 295 300

Phe Tyr Thr Leu Leu Thr Leu Leu Pro Thr Tyr Met Lys Glu Val Leu 305 310 310 315

Arg Phe Asn Ile Gln Glu Asn Gly Phe Leu Ser Ala Val Pro Tyr Leu 325 330 335

Gly Cys Trp Leu Cys Met Ile Leu Ser Gly Gln Ala Ala Asp Asn Leu 340 345 350

Arg Ala Arg Trp Asn Phe Ser Thr Leu Trp Val Arg Arg Val Phe Ser 355 360 365

Leu Ile Gly Met Ile Gly Pro Ala Ile Phe Leu Val Ala Ala Gly Phe 370 375 380

Ile Gly Cys Asp Tyr Ser Leu Ala Val Ala Phe Leu Thr Ile Ser Thr 385 390 395 400

Thr Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Asp 405 410 415

Ile Ala Pro Ser Tyr Ala Gly Ile Leu Leu Gly Ile Thr Asn Thr Phe 420 425 430

Ala Thr Ile Pro Gly Met Ile Gly Pro Ile Ile Ala Arg Ser Leu Thr 435 440 445

Pro Glu Asn Thr Ile Gly Glu Trp Gln Thr Val Phe Cys Ile Ala Ala 450 455 460

Ala Ile Asn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly
465 470 475 480

Glu Val Gln Asn Trp Ala Ile Ser Asp His Gln Gly His Arg Asn 485 490 495

<210> 5

```
<211> 31
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: PCR primer
<400> 5
cgggatcccg ccngcnatgc ayrshrtstg g
                                                                    31
<210> 6
<211> 29
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: PCR primer
<400> 6
ggaattccdg gdgcratktc narrtrrtt
                                                                    29
<210> 7
<211> 2930
<212> DNA
<213> Homo sapiens
<220>
<221> CDS
<222> (263)..(1870)
<400> 7
gttcggtcga agccctcccc ttaattatgt gcaattcaag tccccactgc ccgcccgcaa 60
geocceacte atectegetg egggeagggt ggeocctgea etttacaagg gggtgeagga 120
gcgggagacg gtcgtccgaa caccggctcc ccggcatgcg tagaccggcg ggcggagcgg 180
geteactitg egecaateet acgagaacte ceagaactee getteectag tecaacceaa 240
gecagagiting cocacaceta ag ath ged ged ged ged ath aca eeg eee ege 292
                         Met Ala Ala Gly Ala Met Thr Pro Pro Arg
```

15

ccg gtc cag cca gct cgg ccc ggg ggc ttc ggg ctg tcg ggc cgg cgc Pro Val Gln Pro Ala Arg Pro Gly Gly Phe Gly Leu Ser Gly Arg Arg

20

tcc	CE	cto	t g	c ca	g gt	g gc	g ag	t ac	a cc	t go	t ca	ic at	a go	ic at	cat	g 388
Ser	Let	ı Let	ı Cy	s Gl	n Va	1 A1	a Se	r Th	r Pr	o Al	a Hi	s Va	1 61	. V V=	l Me	-
			. 3					3						10	-1 110	•
	-							•	•				4			
agg	tet		י מד		a											
722	501	e Dec	, y.	ו אב	a ya	- •	g gc	e eg	y aa	c ga	r gg	c ga	g ga	g ag	c ac	g 436
Arg	361			I AI	g AS	p Le			g As	n As	p G1	y Gl	u Gl	u Se	r Th	r
		4.5	>				5	0				5	5			
														•		
gac	cgo	acg	1 cc	t ct	t ct	a cc	g g g	c gc	c cc	a cg	g gc	c ga	a gc	c gc	t cca	484
Asp	Arg	J Thi	Pro	o Le	u Le	u Pro	o Gly	y Al	a Pr	o Ar	g Al	a Gl	u Al	a Al	a Pro	.
	60					6					7					
gtg	tgo	: tgc	: tct	gct	cg1	t tac	aa a	: tta	a gc	a ati	t tto	a ac	- ++		t ggt	
Val	Cys	Cvs	Sei	. Ala	a Arc	TVI	r Ası	Lei	ı Ala	3 T14	بعد ا اها ه	. Δ1:	a Dh	o Dh	e Gly	532
75	•	•			80					8.9		u Ale	a Pir	C PI	-	
					•	•				0.	•				90)
**	++-								•							
Dha	Dha	. all	. 9.00	, Lai	. gc		e egt	. gcc	aat	CEC	gagt	gtt	ge	g tta	a gtg	580
Pile	Pne	: iie	val			L Let	1 Arg	, val			ı Sei	r Val	Ala	a Le	u Val	
				95	•				100)				10	5	
															aag	628
Asp	Met	Val	Asp	Ser	Asn	Thr	Thr	Leu	Glu	Asp	Asr.	Arg	Thi	Sez	Lys	
			110					115	,				120)	-	
gcg	tgt	cca	gag	cat	tct	gct	ccc	ata	aaa	qtt	cat	cat	aat	: caa	acg	676
															Thr	070
	•	125					130		-,-			135		. G11.	. 1111	
												433				
aat .	aac	ааσ	tac	caa	taa	ant.	~~=	~	201						ggt	
																724
G ₁	140	Lys	ıyı	GIII	ırp		MIG	GIU	inr	GIN		lib	IIe	Leu	Gly	
	TAO					145		*			150					
												gga				772
	Phe	Phe	Tyr	Gly	Tyr	Ile	Ile	Thr	Gln	Ile	Pro	Gly	Gly	Tyr	Val	
155					160					165					170	
gcc	agc	aaa	ata	ggg	9 99	aaa	atg	ctg	cta	gga	ttt	999	atc	ctt	ggc	820
Ala	Ser	Lys	Ile	Gly	Gly	Lys	Met	Leu	Leu	Gly	Phe	Gly	Ile	Leu	Glv	
				175					180	-		•		185		
														-00		
act	gct	qtc	ctc	acc	cta	ttc	act	ccc	att	act	acs	gat	tt=		~++	060
Thr	Ala	Val	Len	Thr	1.611	Dhe	Th-	Dro	T) =	אות	77.	Asp	Lea	gya	y.:	868
			190	****	Dea	£11.C			11E	WIG	wra	wsb		GTÅ	val	
	-		T30		•			195					200			
												gga				916
зтХ			Ile	Val	Leu	Arg	Ala	Leu	Glu	Gly	Leu	Gly	Glu	Gly	Val	
		205					210					215				

									tgg Trp 230				964
_	_								gga Gly				1012
	_								tgc Cys		_		1060
			_						gga Gly				1108
	_								caa Gln		_	-	1156
				-	_				tca Ser 310	-		-	1204
									att Ile				1252
			_		_	-			tac Tyr				1300
			_			_		_	aag Lys				1348
		_		_					ttg Leu				1396
			_	-					gct Ala 390				1444
									aga Arg		Ser		1492

															ttc		1540
	TIE	GIY	Mec	116	415		ATA	vai	. Pne	420		Ala	Ala	Gly	Phe	Ile	
										120					425		
9	ggc	tgt	gat	tat	tct	ttg	gcc	gtt	gct	ttc	cta	act	ata	tca	aca	aca	1588
(Gly	Cys	Asp	Tyr	Ser	Leu	Ala	Val	Ala	Phe	Leu	Thr	Ile	Ser	Thr	Thr	
				430					435	·				440			
	~+ <i>~</i>		~~~		F. 7.0												
															gat Asp		1636
	-	- -,	445		-,-	501	501	450			***	ASII	455	Dea	ASP	TIE	
															ttt		1684
1	Ala		Ser	Tyr	Ala	Gly		Leu	Leu	Gly	Ile	Thr	Asn	Thr	Phe	Ala	
		460					465					470					
á	act	att	сса	gga	ato	att	aaa	ccc	atc	att	gct	aaa	agt	cta	acc	cct	1732
															Thr		1/32
	175					480					485	-				490	
															gct		1780
•	LSp	ASN	Thr	vaı	G1y	GIU	Trp	GIN	Thr	Val	Phe	Tyr	Ile	Ala	Ala	Ala	
					475					300					505		
a	att	aat	gtt	ttt	ggt	gcc	att	ttc	ttt	aca	cta	ttc	gcc	aaa	ggt	gaa	1828
1	le	Asn	Val	Phe	Gly	Ala	Ile	Phe	Phe	Thr	Leu	Phe	Ala	Lys	Gly	Glu	
				510					515					520			
_	rta	C22	aac	+~~	act	ctc	225	73 t	636	a nt	~~~						
			Asn										_				1870
			525					530			- -7		535				
t	gaa	ggaa	icc a	ataa	ataa	t cc	tgcc	tcta	tta	atgt	att	ttta	ttta	tc a	tgta	acctc	1930
		+~~	~	+~+ =	++~+	a ta	3003	++c+	2 + ~	+						ttaga	
-	aag	Lyce		Lyca	cege	y ca	ayca		alg			LLaa	ctgt	ac t	tgta	ttaga	1990
t	ttt	taag	igc c	tata	atca	t ga	aata	tcac	tag	ttgc	cag	aata	ataa	aa t	gaac	tgtgt	2050
				•													
t	taa	ttat	ga a	taat	atgt	a ag	ctag	gact	tct	actt	tag	gttc	acat	ac c	tgcc	tgcta	2110
~		~~ ~ ~			~+ ~~			atat	+								
9	ceg	yyca	ac a	ugaa	ycag	y ac	agtt	cigt	rga		cta	agge	cata	ct a	aagg	gaatg	2170
a	gct	gaaa	ca g	acct	cctg	a ta	cctt	tgct	taai	ttaa	act a	agato	ata:	at t	ctcad	ggtac	2230
	_	-	J					-						_			-
t	gat	aaac	ac c	tgtt	gttg	t tc	actt	tcct	cata	aaaa	att 9	gtcag	gete	tc t	ctgad	cactt	2290
a	gac	ctca	aa c	ttta	gcat	c tc	tgtg	gagc	tgc	catco	cac t	igtat	aatt	it c	gcctg	gcaa	2350

<210> 8

<211> 536

<212> PRT

<213> Homo sapiens

<400> 8

Met Ala Ala Gly Ala Met Thr Pro Pro Arg Pro Val Gln Pro Ala Arg
1 5 10 15

Pro Gly Gly Phe Gly Leu Ser Gly Arg Arg Ser Leu Leu Cys Gln Val 20 25 30

Ala Ser Thr Pro Ala His Val Gly Val Met Arg Ser Pro Val Arg Asp 35 40 45

Leu Ala Arg Asn Asp Gly Glu Glu Ser Thr Asp Arg Thr Pro Leu Leu 50 55 60

Pro Gly Ala Pro Arg Ala Glu Ala Ala Pro Val Cys Cys Ser Ala Arg
65 70 75 80

Tyr Asn Leu Ala Ile Leu Ala Phe Phe Gly Phe Phe Ile Val Tyr Ala 85 90 95

Leu Arg Val Asn Leu Ser Val Ala Leu Val Asp Met Val Asp Ser Asn 100 105 110

Thr Thr Leu Glu Asp Asn Arg Thr Ser Lys Ala Cys Pro Glu His Ser 115 120 125

- Ala Pro Ile Lys Val Lis His Asn Gln Thr Gly Lys Lys Tyr Gln Trp 130 135 140
- Asp Ala Glu Thr Gln Gly Trp Ile Leu Gly Ser Phe Phe Tyr Gly Tyr 145 150 155 160
- Ile Ile Thr Gln Ile Pro Gly Gly Tyr Val Ala Ser Lys Ile Gly Gly
 165 170 175
- Lys Met Leu Gly Phe Gly Ile Leu Gly Thr Ala Val Leu Thr Leu 180 185 190
- Phe Thr Pro Ile Ala Ala Asp Leu Gly Val Gly Pro Leu Ile Val Leu 195 200 205
- Arg Ala Leu Glu Gly Leu Gly Glu Gly Val Thr Phe Pro Ala Met His 210 215 220
- Ala Met Trp Ser Ser Trp Ala Pro Pro Leu Glu Arg Ser Lys Leu Leu 225 230 235 240
- Ser Ile Ser Tyr Ala Gly Ala Gln Leu Gly Thr Val Ile Ser Leu Pro 245 250 255
- Leu Ser Gly Ile Ile Cys Tyr Tyr Met Asn Trp Thr Tyr Val Phe Tyr
 260 265 270
- Phe Phe Gly Thr Ile Gly Ile Phe Trp Phe Leu Leu Trp Ile Trp Leu 275 280 285
- Val Ser Asp Thr Pro Gln Lys His Lys Arg Ile Ser His Tyr Glu Lys 290 295 300
- Glu Tyr Ile Leu Ser Ser Leu Arg Asn Gln Leu Ser Ser Gln Lys Ser 305 310 315 320
- Val Pro Trp Val Pro Ile Leu Lys Ser Leu Pro Leu Trp Ala Ile Val 325 330 335
- Val Ala His Phe Ser Tyr Asn Trp Thr Phe Tyr Thr Leu Leu Thr Leu 340 345 350
- Leu Pro Thr Tyr Met Lys Glu Ile Leu Arg Phe Asn Val Gln Glu Asn 355 360 365

Gly Phe Leu Ser Ser Leu Pro Tyr Leu Gly Ser Trp Leu Cys Met Ile 370 380

Leu Ser Gly Gln Ala Ala Asp Asn Leu Arg Ala Lys Trp Asn Phe Ser 385 390 395 400

Thr Leu Cys Val Arg Arg Ile Phe Ser Leu Ile Gly Met Ile Gly Pro 405 410 415

Ala Val Phe Leu Val Ala Ala Gly Phe Ile Gly Cys Asp Tyr Ser Leu
420 425 430

Ala Val Ala Phe Leu Thr Ile Ser Thr Thr Leu Gly Gly Phe Cys Ser 435 440 445

Ser Gly Phe Ser Ile Asn His Leu Asp Ile Ala Pro Ser Tyr Ala Gly
450 455 460

Ile Leu Leu Gly Ile Thr Asn Thr Phe Ala Thr Ile Pro Gly Met Val 465 470 475 480

Gly Pro Val Ile Ala Lys Ser Leu Thr Pro Asp Asn Thr Val Gly Glu 485 490 495

Trp Gln Thr Val Phe Tyr Ile Ala Ala Ile Asn Val Phe Gly Ala
500 505 510

Ile Phe Phe Thr Leu Phe Ala Lys Gly Glu Val Gln Asn Trp Ala Leu
515 520 525

Asn Asp His His Gly His Arg His 530 535

<210> 9

<211> 1485

<212> DNA

<213> Artificial Sequence

<220>

<220>

<221> CDS

<222> (1)..(1485)

< 400																
											gay				_	48
Met	Xaa	Xaa	Pro	Val	Xaa	Asp	Xaa	Ala	Xaa	Xaa	Xaa	Gly	Glu	Glu	Xaa	
1				5					10					15		
						•										
wcg	gac	cgc	acr	cck	cty	ctr	cmg	sgc	gcc	ccr	cgg	gcs	gaa	scc	qct	96
_	-	_									Arg	-	_		-	
			20					25					30			
													•			
cca	atr	tac	tac	tct	act	cat	tac	aac	vta	σca	wtt	tta	kcc		+++	14
	_										Xaa	_				44
	nu.	35	-7-				40		••••			45				
												43				
cat	++0	++~	-	ete	tat	kca	tta	cak	ata	aat	ctg	am.	~++	~~~		10
											Leu		-	_	-	19:
gry		FILE	Add	nad	- Y -	55	TEG	AGG	vai	waii		Add	vai	Add	vqq	
	50					33					60					
					+ = =	221/	262	201	k		gat			1-		24
		_									Asp		_			24
-	хаа	met	Add	ASP		Aaa	1111	1111	vaq		ASP	ASII	Arg	xaa		
65					70					75					80	
																200
		_									gtt		_	_		28
Xaa	Xaa	Cys	Xaa		Hls	ser	Ala	Pro		Lys	Val	Xaa	Xaa		Gln	
				85					90					95		
_			_					_	_		caa					336
Thr	Gly	Xaa	Lys	Tyr	Xaa	Trp	Asp	Ala	Glu	Thr	Gln	Gly	Trp	Ile	Leu	
			100					105					110			
ggw	tcy	ttt	tty	tat	ggc	tac	atc	atc	aca	car	att	cct	gga	gga	tat	384
Xaa	Xaa	Phe	Xaa	Tyr	Gly	Tyr	Ile	Ile	Thr	Xaa	Ile	Pro	Gly	Gly	Tyr	
		115					120					125				
gtt	gcc	agc	ara	akw	9 99	99 9	aar	mtg	ytg	cta	gga	tty	999	atc	ytt	432
Val	Ala	Ser	Xaa	Xaa	Gly	Gly	Xaa	Xaa	Xaa	Leu	Gly	Xaa	Gly	Ile	Xaa	
	130					135					140					
gsy	acw	gct	rtc	ytc	acc	ctg	ttc	act	ccc	mty	gct	gca	gat	ttm	gga	480
-		_		_							Ala					
145					150					155					160	
-																
gty	gga	scm	cty	rtt	gya	ctc	agr	gca	cta	gaa	ggr	cta	gga	gag	ggt	528
											Xaa					
	,			165					170				•	175	•	
									. •							
atv	aca	twt	CCA	acc	atσ	cat	acc	ato	tga	tct	tcw	taa	act	ccc	cct	576
											Xaa					3.0
vaq	TITE	Add	190	~_a	,-, , ,-			185					190			

															r ctt	
Leu	Glu	Arg	Ser	Xaa	a Let	ı Xaa	a Xaa	a Ile	. Xa	а Ту	r Ala	a Gly	/ Al	a Xa	a Leu	
		199	i				200)				205	5			
	'															
9 99	aca	gta	rtt	tct	ctt	cct	ctt	tct	gga	a rt	a att	tgo	ta	c ta	t atg	672
															r Met	
_	210					215				•	220			7		
aat	taa	act	tat	qto	tto	: tav	/ ttv	, ttt	gan	/ avi	t rtt	: œa	atr	n whi	y tgg	720
															Trp	
225			•		230					23!		,		· nac	240	
															240	
ttt	mtt	ttr	taa	ato	tas	tta	att	agt	gav	, aca	. cca		2001		aag	260
															Lys	768
		1144		245			· val		250		. FIC	, Add	Add		-	
				243	,				250	,	-			255	•	
262	atv	west	- CMV	+=+	~~~	220	~~~	+=17	2++	at t		+			aat	
															aat Asn	816
Add	naa	Add	260		GIU	шys	лаа		116	Det	. Ser	261			ASN	
			260					265					270	,		
													_		tcm	864
GIII	лаа	275	Ser	GIII	гуз	Ser		PIO	Trp	Add	лаа		хаа	Lys	Xaa	
		2/5					280			-		285				
					aty											912
Leu		Leu	urp	Ala	хаа		vaı	Ala	хаа	Pne		тут	Asn	Trp	Thr	
	290					295					300					
					acm						_	_	_			960
	Tyr	inr	xaa	Leu	Xaa	Leu	ren	Pro	inr		met	Lys	xaa	Xaa		
305					310					315					320	
					gag											1008
Arg	Phe	Asn	Xaa		Glu	Asn	GIA	Phe		Ser	Xaa	Xaa	Pro	Tyr	Leu	
				325					330					335		
					atg								_			1056
Xaa	Xaa	Trp		Cys	Met	Ile	Leu		Gly	Gln	Ala	Ala	Asp	Asn	Leu	
			340					345					350			
					ttt										_	1104
Arg	Ala	Xaa	Trp	Asn	Phe	Ser	Thr	Xaa	Xaa	Val	Xaa	Arg	Xaa	Phe	Ser	
		35 5					360					365				
ctt	ata	ggr	atg	att	gga	cct	gcr	rta	ttc	ctg	gtw	gcy	gcw	ggm	tty	1152
Leu	Ile	Xaa	Met	Ile	Gly	Pro	Xaa	Xaa	Phe	Leu	Xaa	Xaa	Xaa	Xaa	Xaa	
	370					275					200					

	ggc	tgt	gat	tat	tcy	ttg	gcy	gtt	gcw	ttc	cta	acy	ata	tca	aca	1200
Xaa	Gly	Cys	Asp	Tyr	Xaa	Leu	Xaa	Val	Xaa	Phe	Leu	Xaa	Ile	Ser	Thr	
385					390					395					400	
	-															
acm	ctg	gga	ggc	ttt	tgc	tct	tct	gga	ttt	agc	atc	aac	cat	ctg	gay	1248
Xaa	Leu	Gly	Gly	Phe	Cys	Ser	Ser	Gly	Phe	Ser	Ile	Asn	His	Leu	Xaa	
				405	-				410					415		
att	gct	cct	tcg	tat	gct	ggt	aty	ctc	ctg	ggc	atc	aca	aat	acm	ttt	1296
							Xaa									
			420	_		_		425		-			430			
qcc	act	att	ccw	gga	atq	rtt	999	ccc	rtc	att	qcy	ara	agt	ctk	acc	1344
_					_		Gly						_			
	_			-			•									
		435					440					445				
		435					440				•	445				
cct	gak		act	rtt	gga	qaa		caa	acv	gtk	ttc		atv	gct	gct	1392
	-	aac				_	tgg		-	-		try	•	-	~	1392
	Xaa	aac				_			-	-	Phe	try	•	-	~	1392
	-	aac				Glu	tgg		-	-		try	•	-	~	1392
Pro	Xaa 450	aac Asn	Thr	Xaa	Gly	Glu 455	tgg Trp	Gln	Xaa	Xaa	Phe 460	try Xaa	Xaa	Ala	Ala	
Pro gct	Xaa 450 aty	aac Asn	Thr gtw	Xaa ttt	Gly	Glu 455 gcc	tgg Trp	Gln ttc	Xaa	Xaa	Phe 460 cta	try Xaa ttc	Xaa gcc	Ala aaa	Ala ggt	1392
Pro gct Ala	Xaa 450 aty	aac Asn	Thr gtw	Xaa ttt	Gly ggt Gly	Glu 455 gcc	tgg Trp	Gln ttc	Xaa	Xaa aca Thr	Phe 460 cta	try Xaa ttc	Xaa gcc	Ala aaa	Ala ggt Gly	
Pro gct	Xaa 450 aty	aac Asn	Thr gtw	Xaa ttt	Gly	Glu 455 gcc	tgg Trp	Gln ttc	Xaa	Xaa	Phe 460 cta	try Xaa ttc	Xaa gcc	Ala aaa	Ala ggt	
gct Ala 465	Xaa 450 aty Xaa	aac Asn aat Asn	Thr gtw Xaa	Xaa ttt Phe	ggt Gly 470	Glu 455 gcc Ala	tgg Trp att	Gln ttc Phe	Xaa tty Xaa	Xaa aca Thr 475	Phe 460 cta Leu	try Xaa ttc Phe	gcc Ala	Ala aaa Lys	Ala ggt Gly	1440
gct Ala 465	Xaa 450 aty Xaa gtr	aac Asn aat Asn	Thr gtw Xaa	Xaa ttt Phe	Gly ggt Gly 470	Glu 455 gcc Ala mtc	tgg Trp att Ile	Gln ttc Phe	Xaa tty Xaa cac	Xaa aca Thr 475	Phe 460 cta Leu	try Xaa ttc Phe	gcc Ala	Ala aaa Lys mac	Ala ggt Gly	
gct Ala 465	Xaa 450 aty Xaa gtr	aac Asn aat Asn	Thr gtw Xaa	Xaa ttt Phe	Gly ggt Gly 470	Glu 455 gcc Ala mtc	tgg Trp att	Gln ttc Phe	Xaa tty Xaa cac	Xaa aca Thr 475	Phe 460 cta Leu	try Xaa ttc Phe	gcc Ala	Ala aaa Lys mac	Ala ggt Gly	1440

<210> 10

<211> 495

<212> PRT

<213> Artificial Sequence

<400> 10

Met Xaa Xaa Pro Val Xaa Asp Xaa Ala Xaa Xaa Xaa Gly Glu Glu Xaa 1 5 10 15

Xaa Asp Arg Xaa Xaa Xaa Xaa Xaa Ala Xaa Arg Xaa Glu Xaa Ala 20 25 30

Pro Xaa Cys Cys Ser Ala Arg Tyr Asn Xaa Ala Xaa Leu Xaa Phe Phe 35 40 45

Gly Phe Phe Xaa Xaa Tyr Xaa Leu Xaa Val Asn Leu Xaa Val Xaa Xaa 50 55 60

Val Xaa Met Xaa Asp Ser Xaa Thr Thr Xaa Xaa Asp Asn Arg Xaa Ser 65 70 75 80

- Xaa Xaa Cys Xaa Glu His Ser Ala Pro Ile Lys Val Xaa Xaa Xaa Gln
 85 90 95
- Thr Gly Xaa Lys Tyr Xaa Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu 100 105 110
- Xaa Xaa Phe Xaa Tyr Gly Tyr Ile Ile Thr Xaa Ile Pro Gly Gly Tyr
 115 120 125
- Val Ala Ser Xaa Xaa Gly Gly Xaa Xaa Xaa Leu Gly Xaa Gly Ile Xaa 130 135 140
- Xaa Gly Xaa Xaa Xaa Leu Xaa Ala Leu Glu Xaa Leu Gly Glu Gly
 165 170 175
- Xaa Thr Xaa Pro Ala Met His Ala Met Trp Ser Xaa Trp Ala Pro Pro 180 185 190
- Leu Glu Arg Ser Xaa Leu Xaa Xaa Ile Xaa Tyr Ala Gly Ala Xaa Leu 195 200 205
- Gly Thr Val Xaa Ser Leu Pro Leu Ser Gly Xaa Ile Cys Tyr Tyr Met 210 215 220
- Asn Trp Thr Tyr Val Phe Xaa Xaa Phe Xaa Xaa Xaa Gly Xaa Xaa Trp 225 230 235 240
- Phe Xaa Xaa Trp Ile Xaa Leu Val Ser Xaa Thr Pro Xaa Xaa His Lys
 245 250 255
- Xaa Xaa Xaa Xaa Tyr Glu Lys Xaa Xaa Ile Leu Ser Ser Leu Xaa Asn 260 265 270
- Gln Xaa Ser Ser Gln Lys Ser Val Pro Trp Xaa Xaa Xaa Xaa Lys Xaa 275 280 285
- Leu Pro Leu Trp Ala Xaa Xaa Val Ala Xaa Phe Ser Tyr Asn Trp Thr 290 295 300
- Phe Tyr Thr Xaa Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xaa Leu 305 310 315 320

Arg Phe Asn Xaa Gln Glu Asn Gly Phe Leu Ser Xaa Xaa Pro Tyr Leu 325 330 335

Xaa Xaa Trp Leu Cys Met Ile Leu Xaa Gly Gln Ala Ala Asp Asn Leu 340 345 350

Arg Ala Xaa Trp Asn Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser 355 360 365

Leu Ile Xaa Met Ile Gly Pro Xaa Xaa Phe Leu Xaa Xaa Xaa Xaa Xaa Xaa 370 380

Xaa Gly Cys Asp Tyr Xaa Leu Xaa Val Xaa Phe Leu Xaa Ile Ser Thr 385 390 395 400

Xaa Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Xaa 405 410 415

Ile Ala Pro Ser Tyr Ala Gly Xaa Leu Leu Gly Ile Thr Asn Xaa Phe 420 425 430

Ala Thr Ile Xaa Gly Met Xaa Gly Pro Xaa Ile Xaa Xaa Ser Xaa Thr 435 440 445

Pro Xaa Asn Thr Xaa Gly Glu Trp Gln Xaa Xaa Phe Xaa Xaa Ala Ala 450 455 460

Ala Xaa Asn Xaa Phe Gly Ala Ile Phe Xaa Thr Leu Phe Ala Lys Gly 465 470 475 480

Glu Xaa Gln Asn Trp Xaa Xaa Xaa Asp His Xaa Gly His Arg Xaa 485 490 495

<210> 11

<211> 1485

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: human/sheep consencus sequence

<220>

<221> CDS

<222> (1) .. (1485)

<400> 11

atg	ang	tcn	ccg	gtt	תתת	gac	ntn	gcc	cng	anc	gan	ggc	gag	gag	ngc	48
Met	Xaa	Xaa	Pro	Val	Xaa	Asp	Xaa	Ala	Xaa	Xaa	Xaa	Gly	Glu	Glu	Xaa	
1				5					10)				15		
nca	σač	cac	acn	CCB	ctn	ctn	cna	חמכ	acc	CCD	caa	acn	722	ncc	gct	96
_	_											-	-		Ala	96
Add	ASD	Arg		лаа	Add	παα	Add		A1 a	Naa	Arg	Add			Ala	
			20					25					30			
	_	_	-					aac		_		_				144
Pro	Xaa	Cys	Cys	Ser	Ala	Arg	Tyr	Asn	Xaa	Ala	Xaa	Leu	Xaa	Phe	Phe	
		35					40					45				
ggt	ttc	ttc	ntt	ntn	tat	nca	tta	cgn	gtg	aat	ctg	agn	gtt	gen	nta	192
Glv	Phe	Phe	Xaa	Xaa	Tyr	Xaa	Leu	Xaa	Val	Asn	Leu	Xaa	Val	Xaa	Хаа	
	50		-		•	55				_	60					
	-															
					+	225	202									240
	-	_	_	_				act			-		-			240
	хаа	Met	хаа	Asp		лаа	Inr	Thr	хаа		-	ASI	Arg	xaa	_	
65					70					75					80	
											•					
		-						CCC			_					288
Xaa	Xaa	Cys	Xaa	Glu	His	Ser	Ala	Pro	Ile	Lys	Val	Xaa	Xaa	Xaa	Gln	
				85					90					95		
acg	ggt	aan	aag	tac	cnn	tgg	gat	gca	gaa	act	caa	gga	tgg	att	ctc	336
Thr	Glv	Xaa	Lvs	Tyr	Xaa	Trp	Asp	Ala	Glu	Thr	Gln	Gly	Trp	Ile	Leu	
			100	•		•	•	105				•	110			
~~~	+		++5	+=+	996	tac	atc	atc	202	can	a++	cct	<b>772</b>	<i>aa</i> =	tat	384
																304
лаа	лаа		Add	ıyı	GLY	ıyı		Ile	IIII	Add	116		GIY	GIY	TYL	
		115					120					125				
-	-	_						ntg	_							432
Val	Ala	Ser	Xaa	Xaa	Gly	Gly	Xaa	Xaa	Xaa	Leu	Gly	Xaa	Gly	Ile	Xaa	
	130					135					140					
gnn	acn	gct	ntc	ntc	acc	ctg	ttc	act	CCC	ntn	gct	gca	gat	ttn	gga	480
Xaa	Xaa	Ala	Xaa	Xaa	Thr	Leu	Phe	Thr	Pro	Xaa	Ala	Ala	Asp	Xaa	Gly	
145					150					155					160	
atn	gga	nen	ctn	ntt	gna	ctc	acm	gca	cta	gaa	gan	CLA	да	gag	gat	528
_								Ala								
Add	GLY	Add	AUG	165				<i>-</i> a	170		3044		7	175	1	
				703					1,0					-13		
									<b>.</b>							
gtn		rn-					~~~									576
				_											•	3,0
Xaa				_				Met 185				Trp			•	3,0

ctt	gaa	aga	ago	aan	ctt	ctr	agn	att	tcr	ta	ge	a gga	gca	acar	ctt	624
Leu	Glu	Arg	Ser	Xaa	Leu	Хаа	Xaa	ılle	xaa	Ty	r Ala	a Gly	/ Ala	a Xaa	. Leu	
		195					200	)				205	5			
	_															
999	aca	gta	ntt	tct	ctt	cct	ctt	tct	gga	nta	att	tqc	tac	tat	atg	672
															Met	• • •
•	210					215			•		220	_	- 7 -	/ -		
														•		
aat	taa	act	tat	arc	ttc	tan	ttr	ttt	gar	ant	ntt	gga	atr	n - n	tgg	720
															Trp	720
225			-,-		230					235		. 017		. Aud	240	
															240	
<b>+++</b>	ntt	ttn	taa	atc	tan	tta	att	agt	gan	aca	CCA	naa	200		aaq	760
															Lys	768
- 110	Aua	Add	110	245		neu		Jer	250		PIO	лаа	Add		-	
		•		243					250					255		
202	250	202	655		<b>~</b> ~~	224		+	<b></b>	a++	+			ana		
											-				Asn	816
Add	лаа	naa	260	ıyı	GIU	Lys	Aaa	265	116	Leu	. ser	ser			ASI	
			200					207					270			
c= <b>c</b>	ctn	tat	<b>.</b>	~~~	330	+ = =	ata		taa	<b>n</b> +-	222	250		aaa	<b>.</b>	064
														Lys		864
GIII	Aaa	275	SEL	GIII	цуз	Ser	280	PIO	ΙΙĐ	Ada	лаа		Add	Lys	Xaa	
		213					200					285				
cta	cca	ctt	taa	act	atn	at n	att	GC3	can		tat	* > 0	225	tgg		010
														Trp		912
Deu	290	Deu	ııp	AIG	vaa	295	VAI	ALA	Ada	FIIC	300	TYL	ASII	пр	Int	
	230					233					300					
	tat	act	ttn	tta	2 CD	tta	tta	cct	act	tan	ata	224	~an	ntc		960
												_	_	Xaa		960
305	-7-	****	Aud	200	310	Dea	200	710		315	1166	Lys	AGG	Add		
303					310					212					320	
200	ttc	22F	ntt	caa	asa.	22 <b>t</b>	aaa	+++	tta	tet	nca	ntn	cct	tat	•••	1008
					-									Tyr		1008
~-9	FIIG	A3II	Aua	325	GIU	<b></b>	GLY	FIIC	330	Jei	AGG	Add	PIO	335	Deu	
				323					330					333	•	
aan	tnt	taa	tta	tat	ato	atc	cta	ten	aat	caa	act	act	asc.	aat	tta	1056
				_	_		_				_	_	_	Asn		1000
Auu	nuu		340	Cys	1100	***	200	345	01,	<b>V</b>	744	~~u	350	7011	Deu	
			340					343					330			
=aa	aca	ana	taa	22F		tca	act	ntn	tom	att	com	2072	ntt	+++	300	1104
	-								_	_	_	_		ttt Phe	•	1104
Arg	MIG		TLD	WZII	PHE	SEL		naa	Aaa	, var	лаа		vaa	Pne	Ser	
		355					360					365				
		-								at-	<b>~</b> +~	~~-				1150
			_				_			_	_	_	_	ggn		1152
ren		Add	met	TTE	GTÀ		Add	Add	rne	ren		Add	ABA	Xaa	SBA	
	370					375					380					

														tca		1200
Xaa	Gly	Cys	Asp	Tyr	Xaa	Leu	Xaa	Val	Xaa	Phe	Leu	Xaa	Ile	Ser	Thr	
385					390					395					400	
acn	ctg	gga	ggc	ttt	tgc	tct	tct	gga	ttt	agc	atc	aac	cat	ctg	gan	1248
Xaa	Leu	Gly	Gly	Phe	Cys	Ser	Ser	Gly	Phe	Ser	Ile	Asn	His	Leu	Xaa	
		,		405					410					415		
att	gct	cct	tcg	tat	gct	ggt	atn	ctc	ctg	ggc	atc	aca	aat	açn	ttt	1296
														Xaa		
			420					425		•			430			
gcc	act	att	ccn	gga	atg	ntt	999	ccc	ntc	att	gcn	ana	agt	ctn	acc	1344
														Xaa		
		435					440					445				
cct	gan	aac	act	ntt	gga	gaa	tgg	caa	acn	gtn	ttc	tnn	atn	gct	qct	1392
														Ala		
	450					455					460					
gct	atn	aat	gtn	ttt	ggt	gcc	att	ttc	ttn	aca	cta	ttc	gcc	aaa	ggt	1440
														Lys		
465					470					475				•	480	
gaa	gtn	caa	aac	tgg	gcn	ntc	ant	gat	cac	can	gga	cac	aga	nac		1485
											Gly		-			
				485				-	490		-		_	495		

<210> 12

<211> 495

<212> PRT

<213> Artificial Sequence

<400> 12

Met Xaa Xaa Pro Val Xaa Asp Xaa Ala Xaa Xaa Xaa Gly Glu Glu Xaa l

Xaa Asp Arg Xaa Xaa Xaa Xaa Xaa Ala Xaa Arg Xaa Glu Xaa Ala 20 25 30

Pro Xaa Cys Cys Ser Ala Arg Tyr Asn Xaa Ala Xaa Leu Xaa Phe Phe 35 . 40 45

Gly Phe Phe Xaa Xaa Tyr Xaa Leu Xaa Val Asn Leu Xaa Val Xaa Xaa 50 55 60

Val Xaa Met Xaa Asp Ser Xaa Thr Thr Xaa Xaa Asp Asn Arg Xaa Ser

_	_	
0		

70

75

80

Xaa Xaa Cys Xaa Glu His Ser Ala Pro Ile Lys Val Xaa Xaa Xaa Gln _ 85 90 95

Thr Gly Xaa Lys Tyr Xaa Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu 100 105 110

Xaa Xaa Phe Xaa Tyr Gly Tyr Ile Ile Thr Xaa Ile Pro Gly Gly Tyr
115 120 125

Val Ala Ser Xaa Xaa Gly Gly Xaa Xaa Xaa Leu Gly Xaa Gly Ile Xaa 130 135 140

Xaa Xaa Ala Xaa Xaa Thr Leu Phe Thr Pro Xaa Ala Ala Asp Xaa Gly
145 150 155 160

Xaa Gly Xaa Xaa Xaa Leu Xaa Ala Leu Glu Xaa Leu Gly Glu Gly
165 170 175

Xaa Thr Xaa Pro Ala Met His Ala Met Trp Ser Xaa Trp Ala Pro Pro 180 185 190

Leu Glu Arg Ser Xaa Leu Xaa Xaa Ile Xaa Tyr Ala Gly Ala Xaa Leu 195 200 205

Gly Thr Val Xaa Ser Leu Pro Leu Ser Gly Xaa Ile Cys Tyr Tyr Met 210 215 220

Asn Trp Thr Tyr Val Phe Xaa Xaa Phe Xaa Xaa Xaa Gly Xaa Xaa Trp 225 230 235 240

Phe Xaa Xaa Trp Ile Xaa Leu Val Ser Xaa Thr Pro Xaa Xaa His Lys 245 250 255

Xaa Xaa Xaa Xaa Tyr Glu Lys Xaa Xaa Ile Leu Ser Ser Leu Xaa Asn 260 265 270

Gln Xaa Ser Ser Gln Lys Ser Val Pro Trp Xaa Xaa Xaa Xaa Lys Xaa 275 280 285

Leu Pro Leu Trp Ala Xaa Xaa Val Ala Xaa Phe Ser Tyr Asn Trp Thr 290 295 300

Phe Tyr Thr Xaa Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xaa Leu 305 310 315 320

Arg Phe Asn Xaa Gln Glu Asn Gly Phe Leu Ser Xaa Xaa Pro Tyr Leu

325 330 335

- Xaa Xaa Trp Leu Cys Met Ile Leu Xaa Gly Gln Ala Ala Asp Asn Leu
   340 345 350
- Arg Ala Xaa Trp Asn Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser 355 360 365
- Leu Ile Xaa Met Ile Gly Pro Xaa Xaa Phe Leu Xaa Xaa Xaa Xaa Xaa 370 375 380
- Xaa Gly Cys Asp Tyr Xaa Leu Xaa Val Xaa Phe Leu Xaa Ile Ser Thr 385 390 395 400
- Xaa Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Xaa 405 410 415
- Ile Ala Pro Ser Tyr Ala Gly Xaa Leu Leu Gly Ile Thr Asn Xaa Phe 420 425 430
- Ala Thr Ile Xaa Gly Met Xaa Gly Pro Xaa Ile Xaa Xaa Ser Xaa Thr 435 440 445
- Pro Xaa Asn Thr Xaa Gly Glu Trp Gln Xaa Xaa Phe Xaa Xaa Ala Ala 450 460
- Ala Xaa Asn Xaa Phe Gly Ala Ile Phe Xaa Thr Leu Phe Ala Lys Gly 465 470 475 480
- Glu Xaa Gln Asn Trp Xaa Xaa Xaa Asp His Xaa Gly His Arg Xaa 485 490 495

International Application No

		1 J / US 99	/166/6
A. CLASSII IPC 7	FICATION OF SUBJECT MATTER C12N15/12 C07K14/705 G01N33,	/50 A61K38/17 //C0	7K16/28
	international Patent Classification (IPC) or to both national classi	fication and IPC	
	SEARCHED		
IPC 7	cumentation searched (classification system followed by classific C12N C07K G01N A61K	ation symbols)	
	ion searched other than minimum documentation to the extent tha		
Electronic d	ata base consulted during the international search (name of data i	base and, where practical, search terms used	a)
C. DOCUME	ENTS CONSIDERED TO BE RELEVANT		
Category 7	Citation of document, with indication, where appropriate, of the	relevant passages	Relevant to claim No.
X	DATABASE EMBL - EMEST20 'Online Entry HS1173506, Acc.no. AA2585 19 March 1997 (1997-03-19) HILLIER, L. ET AL.: "zr59d01.r1 NhHMPu S1 Homo sapiens cDNA clo	13, Soares ne 667681	1,4-6
	5' similar to TR:G507415 G507411 SPECIFIC NA+-DEPENDENT INORGANION COTRANSPORTER." XP002121520 the whole document	5 BRAIN C PHOSPHATE	
		-/	
X Funt	ner documents are listed in the continuation of box C.	Patent family members are listed	in annex.
1	tegories of cited documents :	T later document published after the inte or priority date and not in conflict with	
consid "E" earlier of filling d	ered to be of particular relevance socument but published on or after the international	cited to understand the principle or the invention  X* document of particular relevance, the cannot be considered novel or cannot be.	laimed invention be considered to
which	is cited to establish the publication date of another in or other special reason (as specified) and referring to an oral disclosure, use, exhibition or	involve an inventive step when the do "Y" document of particular relevance; the ocannot be considered to involve an in- document is combined with one or moments, such combination being obvio-	claimed invention ventive step when the ore other such docu-
P docume	ent published prior to the international filling date but nan the priority date claimed	in the art.  3" document member of the same patent	·
Date of the	actual completion of the international search	Date of mailing of the international se-	arch report
5	November 1999	17/11/1999	
Name and n	nailing address of the ISA  European Patent Office, P.8, 5818 Patentiaan 2  NL - 2280 HV Rijswijk	- Authorized officer	
	Tel. (-31-70) 340-2040. Tx. 31 651 epo ni. Fax: (+31-70) 340-3016	Smalt, R	

2

International Application No

		Fui/US 99/16676
	ation) DOCUMENTS CONSIDERED TO BE RELEVANT	
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HELLEROVIST C G ET AL: "ANTITUMOR EFFECTS OF GBS TOXIN: A POLYSACCHARIDE EXOTOXIN FROM GROUP B BETA-HEMOLYTIC STREPTOCOCCUS" JOURNAL OF CANCER RESEARCH AND CLINICAL ONCOLOGY, vol. 120, no. 1/02, 1 January 1993 (1993-01-01), pages 63-70, XP000749401 ISSN: 0171-5216 the whole document	1-20.23, 25.26, 28-40
Y	GEARING, D.P. ET AL.: "Expression cloning of a receptor for human granulocyte-macrophage colony-stimulating factor."  EMBO JOURNAL, vol. 8, no. 12, 1989, pages 3667-76, XP002121518 abstract	1-20,23, 25,26, 28-40
Ρ,Χ	FU, C. ET AL.: "Expressional cloning of CM101 receptor gene from mammalian cells." PROCEEDINGS OF THE AMERICAN ASSOCIATION OF CANCER RESEARCH, vol. 40, March 1999 (1999-03), pages 557-Abstr.3677, XP002121519 the whole document	1-15
•		

ternational application No.

PCT/US 99/16676

Boxi	Observations where certain claims wer found unsearchabl (Continuation if it mit if first ship)
This inte	ernational Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X	Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely: see FURTHER INFORMATION SHEET PCT/ISA/210
2. X	Claims Nos.: because they relate to parts of the international Application that do not comply with the prescribed requirements to such an extent that no meaningful international Search can be carried out, specifically:  Claims 24, 39, 40 and 44 could not be searched to completion due to insufficient characterization of the inhibitors in the description.
3.	Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II	Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This Inte	ernational Searching Authority found multiple inventions in this international application, as follows:
1.	As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.	As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.	As only some of the required additional search fees were timely paid by the applicant, this international Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.	No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
	restricted to the invention first mentioned in the claims: it is covered by claims Nos
Remark	The additional search fees were accompanied by the applicant's protest.  No protest accompanied the payment of additional search fees.

International Application No. PCT/US 99 /16676

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 39, and 40 in as far as it pertains to an in vivo method, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Although claim 22, in as far as it relates to a method for use in vivo, is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Claims 24, 39, 40 and 44 could not be searched to completion due to insufficient characterization of the inhibitors in the description.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

		*
	•	
		*
		./ * . 
• .		
		4 · ·
		·
·	· · · · · · · · · · · · · · · · · · ·	

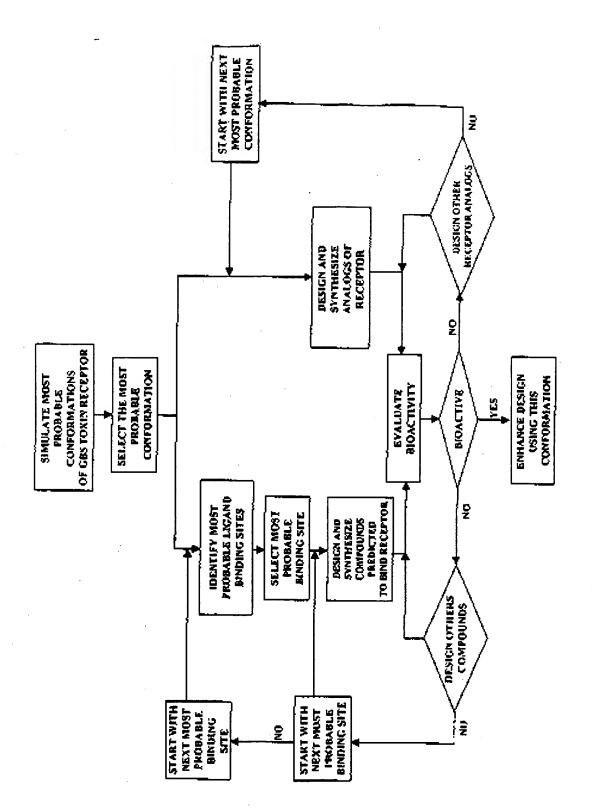












FIG. 3A











### SEQUENCE LISTING

<110:	Fu,	erqv Chan			3										
<120:	• GBS	Toxi	n Re	cept	φr										
< 130	CARE	300-1	/01N	0											
<140:	•			•											
<141	•														
<1502	60-0	93,8	43												
<151>	1998	-07-	22												
<150>	12										_				
<170>	Pate	ntIn	Ver.	. 2.0											
<210>	1														
<211>															
<212>		<b>.</b>													
<213>	nomo	eapı	ens												
<330>															
<221>	CD8														
<222>	(58).	(15	42}												
<400>	1														
teggg	cegge	gctc	actt	ct c	tgcc	aggt	d ac	gagt	acac	ctg	ctca	cgt	agge	gtc	57
atg a	gg tet	ccg	gtt	cga	gac	ctg	gcc	cgg	BAC	gat	<b>3</b> 90	gag	gag	age	105
Met A	rg aez	Pro	Val 5	Arg	Авр	Leu	Ale			ABp	Gly	Glu			
_	*		3					10					15		
ecă di	re ede	acg	cet	ctt	cta	ecg	aac	gcc	CCB	cāā	gee	948	acc	gct	153
Thr A	ip Arg		Pro	Leu	Leu	Pro	Ġly	Ala	Pro	Arg	Ala	Glu	Ala	Ala	
		. 20					25					30			
cca gt	g tgc	tge	tet	gct	cgt	tac	aac	tta	goa	att	ttg	gee	ttt	ttt	201
Pro Va			3er	Ala	Arg	Tyr	ABO	Leu	Ala	Ile	Leu	Ala	Phe	Phe	
	35					40					45				
ggt to	c tto	att	gtg	tat	gca	tta	cgt	gŁg	aat	ctg	#qt	att	aca	tta	249
Gly Pb															-17
	0				55					60					
gt <b>g</b> ga	t atg	gta	gat	tca	aat	aca	act	tţĄ	914	gat	<b>e</b> at	aga	act	tcc	297

Val 65		) Me	t Va	2 AS	p Se 7		n Th	r Th	r Le	با G1 7:		P As:	n Azg	g Th	r Ser 80	
											_					
															caa i Gln	345
-,-				à:		, ,,,,	• ••••		9		» V44.	ı alı	• 47:	99 99		
acg	ggt Glv	aaq Lv:	g aag Lvi	g tad R Tv:	r Gid	e tga	gat Ant	: gca : Ala	e Grae	act ילד ו	caa an	gga Sala	tgg	att	Leu	393
	*",	-,	100					105			· <b>V</b> 11	• 42)	110		: Ten	
ggt gly	Ser Ser	Phe	t Ctt	TY:	- ggc	r tac · Tv:	: atc	: atc	aca Thi	cag : Glr	, ett i lie	- cct	gga Glu	gga nla	tat Tyr	441
		115		,.		-3-	120			_ <b></b> .		125		O. Y	lyr	
att	***	9,00		. a+ :								<b>.</b>			ctt	
															ctt Leu	489
	130					135					140		_			
aa c	act	qct	ato	cto	acc	cta	tte	Act	ccc	att	act	e e	ast	tte		E 3 77
Gly	Thr	Ala	Val	Leu	Thr	Leu	Phe	Thr	Pro	Ile	Ala	Ala	Asp	Leu	Gly	537
145					150			•		155			•		160	
gtt	gga	cca	ctc	att	gta	cts	aga	g¢8	cta	gaz	gga	cta	qqa	<b>9</b> 4ġ	get	585
				Ile					Leu					Olu		***
				165					170					175		
														cce		633
Val	Thr	Phe	Pro 180	Ala	Met	His	Ala		Trp	Ser	Ser	Trp		Pro	Pro	
			160					185					190			
														¢àg		681
Leu	GIA	Arg 195	Sat	Lys	Leu	Leu	9ez 200	Ile	3er	Tyr	Ala	<b>Gly</b> 205	Ala	<b>G</b> ln	Leu	
														tat		729
GLY	210	ANT	TTC	ser	Den	215	Leu	REI	ara	110	220	сув	TYT	Tyx	Met	
			•													
														ttt Pho		777
225		****	2 y 2	7 102	230	• } •		*110	ary	235	TIE	GIĀ	116		77P 260	
					•											
														cac His		825
			- <b>- 6</b> •	245					250				-	255	-y	
<b>.</b>	454	<b>-</b> -	<b></b> -	<b></b>			<b>.</b>	<b>4</b>	<b></b>	<b>.</b>	<b>.</b>	<b>.</b>	<b>.</b>			
94	# LT	ECC	cac	222	age.	443	247	CAC .	att	CEE	TC3	CCB	ETA	aga	<b>aa</b> t	873

Arg	e i 1	5er	260		. Gl	a Ly⊆	; Giu	a Tyr 265		≥ <b>1.6</b> 1	u Se:	r Sei	276		а Авт	
			Ser					Pro					: Let		a tce 5 <b>9e</b> r	921
		Leu					(eV					Тут			act Thr	969
						. Fén					. Net				cta Lou 320	101
					aln					Sex		_			Lta Leu	106.
													•	Asn	tta Leu	111;
											_	_			ear	116
								gta Val		•	•	_	•		ttc Phe	1209
								gtt Val								1257
								gga Gly		_				_	_	1305
								ctc Leu 425		-						1353
	Thr							ece <b>Pro</b>	_		_		_	_		1401
cct	gat	aac	act	gtt	gga	gaa	tgg	CAA	acc	ata	ttc	tat	att	gct	get	1449

Pro Asp Asn Thr Val Gly Glu Trp Gln Thr Val Phe Tyr Ile Ala Ala 450 455 460

gct stt sat gtt ttt ggt gcc att ttt ttt aca cta tto gcc aaa ggt 1497 Ala Ile Abn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly 465 470 475 480

gaa gta cas Aac tgg gct ctc ast gat cac cat ggs cac ags cac

1542
Glu Val Gln Asn Trp Ala Leu Asn Asp His His Gly His Arg His
485
495

tgaaggaaco aataaatast cotgoctota ttaatgtatt tttatttato atgtaacoto 1602 Asagigeett eigiatigig taageatiet aigtetetti tiaatigiae ligiatiaga 1662 tttttaagge etalaateat gaaalateae tagttgeeag aataalaaaa tgaactgtgt 1722 ttaattatga ataatatgta agetaggact tetaetttag geteacatae etgeetgeta 1782 greggyceac atgaagtegg ecagticigt tgatititta gggccatact eeegggsetg 1842 agetgaaaca gaestsetga taestttest taattaaast agatgataat tetsaggtas 1902 tgatesacac ctgttgttgt tcactttoot catasasatt gtcagctotc totgacactt 1962 agaestseae stitageste toigiggage igosalesad igialeatii egestigesa 2022 etggactgag gggagtgtge ccaggeaget gccaageact ceeteectgg ettcagggte 2082 agagigocca gegittatoa gaggoageat coaagoocag agecagigit gactotitegg 2142 ctggtgcctt tcctctgagg ggctatcaat gtgtagataa agccctgagt aggcamgagc 2202 agigagator actgetatyy tottgataca tortcaeact thecettors ageacagagy 2262 astattggct ggcatgceac ctgcassags saastgcgss gcggccgggc acggtggctc 2322 atgeetytaa teeesgeset tigggggget gaggtgggeg aatestgaga teaggagtie 2382 gagaccagee tggccagest ggtgaaacce catetetact aaaaatacsa aaaattaget 2642 gggegtggtg acgggegeet gtaateceag ataeteagga ggetgaggta ggagaateae 2502 ttgaacctgg gaggtggaag ttgcagtgaa ccaagatcac gccactgcac tccagectgg 2562 gratggage pagactecas eteassasa assassas 2602

<210> 2

<211> 495

<212> PRT

<213> Homo eapiens

<400> 2

BASOCCIO- ZALO OCCESTE VITE -

Met Arg Ser Pro Val Arg Asp Leu Ala Arg Asn Asp Gly Glu Glu Ser

1 5 10 15

The Asp Arg Thr Pro Leu Leu Pro Gly Ala Pro Arg Ala Glu Ala Ala 20 25 20

Pro Val Cys Cys Ser Ala Arg Tyr Asn Leu Ala Ile Leu Ala Phe Phe 35 40 45

Gly Phe Phe Ile Val Tyr Ala Leu Arg Val Aen Leu Ser Val Ala Leu So S5 60

Val Asp Met Val Asp Ser Asn Thr Thr Leu Glu Asp Asn Arg Thr Ser 65 70 75 80

Lys Ala Cys Pro Glu Mis Ser Ala Pro île Lys Val His His Asn Gln 85 90 95

Thr Gly Lys Lys Tyr Gln Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu 100 105 110

Gly Sar Phe Phe Tyr Gly Tyr Ile Ile Thr Gln Ile Pro Gly Gly Tyr
115 120 125

Val Ala Ser Lys Ile Gly Gly Lys Met Leu Leu Gly Pha Gly Ile Leu 130 135 140

Gly Thr Ala Val Leu Thr Leu Phe Thr Pro Ile Ala Ala Asp Leu Gly
145 150 155 160

Val Gly Pro Leu Ile Val Leu Arg Ala Leu Glu Gly Leu Gly Gly Gly 165 170 175

Val Thr Phe Pro Ala Met His Ala Met Trp Ser Ser Trp Ala Pro Pro 180 195 190

Leu Glu Arg Ser Lys Leu Leu Ser Ile Ser Tyr Ala Gly Ala Gln Leu 195 200 205

Gly Thr Val Ile Ser Leu Pro Leu Ser Gly Ile Ile Cys Tyr Tyr Met 210 215 220

Asn Trp Thr Tyr Val Phe Tyr Phe Phe Gly Thr Ile Gly Ile Phe Trp 235 240

- Phe Leu Leu Trp Ile Trp Leu Val Ser Amp Thr Pro Gln Lys Him Lym 245 250 255
- Arg lle Ser Rie Tyr Glu Lys Glu Tyr Ile Leu Ser Ser Leu Arg Aen 260 265 270
- Gin Leu Ser Ser Gin Lyë Ser Val Pro Trp Val Pro Ile Leu Lys Ser 275 280 285
- Leu Pro Leu Trp Ala Ile Val Val Ala His Phe Ser Tyr Asn Trp Thr 290 295 300
- Pre Tyr Thr Leu Leu Thr Leu Leu Pro Thr Tyr Met Lys Glu Ils Leu 305 310 315 320
- Arg Phe Asn Vel Glu Asn Gly Phe Leu Ser Ser Leu Pro Tyr Leu 325 330 335
- Gly Ser Trp Leu Cys Net Ile Leu Ser Gly Gln Ala Ala Aep Asn Leu 340 345 350
- Arg Ala Lys Trp Asn Phe Ser Thr Leu Cys Val Arg Arg Ile Phe Ser 355 360 365
- Lou fle Gly Met Ile Gly Pro Ala Val Phe Leu Val Ala Ala Gly Phe 370 375 380
- Ile Gly Cys Asp Tyr Ser Leu Als Val Ala Phe Leu Thr Ile Ser Thr 385 390 395 400
- Thr Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn Ris Leu Asp 405 410 415
- Ile Ala Pro Ser Tyr Ala Gly Ile Leu Leu Gly Ile Thr Asn Thr Phe 420 425 430
- Ala Thr Ile Pro Gly Met Val Gly Pro Val Ile Ala Lys Ser Leu Thr 435 440 445
- Pro Asp Asn Thr Val Gly Glu Trp Gln Thr Val Phe Tyr Ile Ala Ala 450 455 460
- Ala Ile Asn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lys Gly
  465 470 485

Glu Val Glo Asn Trp Ala Leu Asn Asp His His Gly His Arg His 495 495

<210> 3

<211> 2844

<212> DNA

<213> Ovis sp.

<220>

<221> CDS

<222> (84).. (1568)

<400> 3

באפטטטוט אווט יייניבנייבו

configurated dediction dedictored the sales of the configuration of the

egeograget ecctgaagge ate atg aag tee eeg gtt teg gae tta gee eeg 113 Met Lys Ser Pro Val Ser Asp Leu Ala Pro

age gae gge gag gag gge teg gae ege aca eeg ete etg eag ege gee 161 Ser Asp Gly Glu Gly Ser Asp Arg Thr Pro Leu Leu Gln Arg Ala 15 20 25

Fig egg geg gaa doc got dea gta tgo tge tot got egt tad aad eta 209 Pro Arg Ala Glu Pro Ala Pro Val Cys Cys Ser Ala Arg Tyr Asn Leu 30 35 40

gca ttt ttg tcc ttt ttt ggt ttc tte gtt ctc tat tca tta egg gtg 257 Ala Phe Leu Ser Phe Phe Gly Phe Phe Val Leu Tyr Ser Leu Arg Val 45 50 55

Ast ctg age gtt gen eta gtg gae atg gtg gat ten acc aca met gee 305 Asn Leu Ser Val Ala Leu Val Asp Net Val Asp Ser Asn Thr Thr Ala 60 65 70

Axa gat aac aga acg tee tae gag tgt ges gag cat tet get cee ata 350 Lys Asp Asn Arg Thr Ser Tyr Glu Cys Ala Glu His Ser Ala Pro Ile 75 80 85 90

awa gtt ctt can aac caa acg ggt aaa aag tac ogg teg gat gca gaa 401 Lys Val Leu His Asn Gln Thr Gly Lys Lys Tyr Arg Trp Asp Ala Glu 95 100 105

act cas ggs tgg att ctc ggs tct ttt ttc tat ggc tac atc atc aca 449
Thr Gln Gly Trp Ile Leu Gly Ser Phe Phe Tyr Gly Tyr Ile Ile Thr
110 115 120

															y ttg	
Glu	. Ile	Pro	Gly	diy	Typ	c Val	l Als	9 Se 1	. Arg	7 3e1	r Gly	7 31	y Ly:	3 Lei	u Leu	
		125			-		136					13				
nta	785	. Ftc													_	
															- 666	
LEU			OLY	116	Pne	: WT	נתני א	. WT9	: Ile	e Phe			) Phe	Tha	Pro	
	140	)				265	5				150	)				
ata	gct	gca	gat	tto	gga	gto	993	gee	ctt	gtt	: gca	cto	: agg	gca	- cta	593
															Leu	
155					150		-			165			_		170	
							•									
gaa	aaa	cra	on a	(TRA	-	o to			800	0.75	· ata				tgg	
																641
GIH	GTA	LEU	GTÅ			V41	THE	тут			Met	HIS	Ala	Met	Trp	
				175					180					105		
tct	tça	tgg	gct	CCC	cct	ett	gaa	aga	agc	aag	ctt	ctg	agt	att	tea	689
Ser	Ser	Trp	Ala	Pro	Pro	Leu	Glu	Arg	Ser	Lys	Leu	Lau	Ser	Ile	9er	
-			190					195					200			
tat	<b>GCR</b>	GGR	QCB.	CAS	ctt		aca	gta	att	tet	ett	cct	ctt	ter	~~	717
								Val								737
-1-			~~~	244	204	GŁY			AGT	961	DET		men.	per	GIY	
		205					210					215				
							_									
							-	act								785
Val	Ile	Сув	Tyr	Tyr	Met	Asti	<u>dal</u>	Thr	Tyr	Val	Phe	Tyr	Phe	Phe	Gly	
	220					225					230					
att	gtt	gga	atc	4tc	tgg	ttt	att	tta	tgg	atc	tgc	tta	gtt	agt	gat	833
								Leu								
235		_			240				_	245	_				250	
												•				
a ca	CCS	gae.	ach	C2.C	***	**		act			<b>~</b> ~~	7.26	<b>700</b>			400
								Thr								601
****	710	712	4144		-y-	1414	TIE	TIME		171	GT II	րդո	<b>ATM</b>		11e	
				255					360					265		
						_		tet		_	_			_		929
Leu	Ser	9er	Leu	Lyp	Asn	Gln	Leu	9er	Ser	<b>Gl</b> n	Lya	ŝer	Val	Pro	Trp	
			270					275					280			
ata	ect	atg	ctg	AAA	tea	ctg	cca	ctt	tg¤	get	att	atc	att	QCA	cat	977
								Leu		_		_	_	_		
		285					290		P					Men	-11 0	
		ر ه م					4 7 V					295				
	<b></b>						_									
								act								1025
Phe		Tyr	ÀВП	ŢΞĐ			ፓ <u>ሃ</u> ፕ	Thr	Leu	Lev	The	Leu	L¢≀L	διο	Thr	
	DOE					305					310					

tac	atq	g aa	g ga	a gt	C CL	9 <b>9 G</b> G	tt:	c aa	t ati	Ca.	a ga	g set	99	g tt	t tta	1073
Tyr	: Net	Ly	e Gj.	u Va	l Le	n Arg	, Ph	e As:	n Ile	: Gl:	n Gl	u Asr	G)	y Ph	¢ Leu	
315					32					329					330	
									•							
tet	gca	gt	. <b>c</b> o	t ta	t tt	a det	tal	t to	t tta	to:	e ato	z ato	· cto	a to	g ggt	1100
Ser	Ala	. Vai	l Pri	o Tv:	r Lei	u Glv	, LA1	Tri	, 1	L _e rical contraction in the second contract	MAI	y mile	. [ 4.	, Cal	r Gly	1121
				33		~	-,-	,			> 1151		- Pat			
				<b>.</b>	•				340					34	5	
caa	get	gei	. <b>9</b> 80	- 401		a agg	gce	, Tā1	tgg	aet	: tt1	: Cca	act	ct	g tgg	1169
Gln	Ala	) Ala			ı Lei	ı Arg	Ale	Arg	Trp	Asc	Phe	9er	Thr	Lei	J.T.D	
			350	2				355	i				360	}		
gtt	cga	aga	gtt	; ttt	ago	ctt	eta	\$99	atg	att	gga	cet	gcg	ata	tte	1217
Val	Arg	Arg	[Va]	Phe	: Ser	· Leu	Ile	giy	Met	Ile	Gly	Pro	Ala	Ile	Pha	
		365					370					375		787		
												7.2				
cto	att	acc	gra	. 007	+++	ata	990	***	ast	+=+		. 44			gca	
T.411	1741	- או	nla	elv Elv	Dha	714	995	-4-	Ser.	Ta.		. crg	gec	gtt	gca Ala	1265
	380		~~		F114	385	GIA	cys	ASp	172		гел	ΥTS	AUT	Ala	
	300					363					390					
LLC	cta	acc	ata	tca	aca	acc	ctg	88#	<b>3</b> 3c	ttt	tgc	tct	tet	gga	ttt	1313
	Leu	Thr	Ile	Ser	Thr	Thr	FeA	Gly	aly	Phe	Cys	Ser	Ger	Gly	Phe	
395					400					405					410	
																•
agc	atc	820	Cat	ctg	gac	att	gct	cct	tcg	tat	gct	ggt	att	cte	ctg	1361
Ser	Zla	Asn	His	Leu	Aep	Ile	Ala	Pro	Ser	Tyr	Ala	Gly	Ile	Lou	Leu	
				415					420					425		
ggc	atc	ACA	Bat	acc	ttt	gcc	act	att	cct	gga	atq	att	daa	ccc	atc	1409
Gly	Ile	Thr	Aen	Thr	Phe	Ala	Thr	Ila	PTO	Glv	Met	Il.	0.1 v	Þra	Tie	
			430					435					440			
att	GCC.	202	agt	et.t.	acc	cct	ana.	22.5		***						
Tla	Ala	ara	867	TAII	Thr	Pro	alu alu	Ban-	The	#1 -	23	gan.	-99	Caa Cha	act	1657
		445	361	Dea	7111			ABIL	Int	TTE	GIY		IIP	GIN	Thr	
		443					450					455				
	<b>.</b>						_			•						
gtt	cte	tgc	atc	gct	gct	gct	atc	aat	gta	٤٤t	ggt	gcc	att	tt¢	tto	1505
Val	Phe	CAB	Ile	Ala	Ala	Ale .	15¢	Asn	Val	Phe	Gly	Ale	Ţļē	Phe	Phe	
	460					465					470					
aca	cta	ttc	gee	asa	ggt	gaa g	gtg	CRA	aac	tgg	gcc	atc a	agt	gaŁ	CRC	1553
						Glu i										<b>_</b>
475					480					485			– .		490	
					-											
CAA	Q D'A	CAC	ADA	AAC	taar	ggaa	20 2:	at # *	atae:		tata	+a+p	***	a +		1600
			Arg			32-41	di	46	~	نانا	-4-0	LLLA	LLe	~+B.C		16 <b>0</b> B
vei.	7	eir D		A95												
				- T -												

tttgtttatc atgtaaccta amagegoett tgatatttta atgtgtmage amtotatata 1666 casgataaaa bigiaciaga aaaabigigi tagailigia aggobbgtaa bosigaaag 1728 toactagttg coatateage eaasttaget attitteatt attattasce ogthigging 1783 aacttacaat teagggteac atatetgget goaagteagg caacecacaa taggggagtt 1848 ctatitatit ataagaccat acctatagag acgagetgaa atagaccett ctataccitt 1908 gottaattaa ggiggataat aattotoagg tootigttaaa catotgitti igracaeeet 1968 cotcasassa trattigios teagosatos organistas gatoresae triagocios 2028 ccacqqaqet ggcaqccact gtatcattca gcctgqcaac ttcactgagg gaagcatgcc 2088 caggeagetg comeatgtoc cottetetgge tteagggaca gtgeccagea ettaqqoage 2148 atocaagaco agggteageg coaaggettt ggacggtatt ottocootgg ggetgttaat 2200 gtgtggstgs agcootgago caacagggac agogogator acagtoatgg titocatgca 2268 contribect tecchicora gradacigga giatigectg gratgiaacs igcaaaaqaa 2328 agtgtgatge ctaattagee acatataaca teateettga tgateetace tteacatgga 2388 teagagtata aatottemag teetgtgtte taggagotae accagaataa ttaaaatata 2448 aaaageaaca aaecattitt totytotymo acctaagtyt otyyttycay ticawyytta 2508 aagtgactto tacttcacat aacctgcaac oggtggtgta atcatctta gtgttqqttt 2568 ertaaatett attittudag tilliteetgg accalettee agiggittig ageatgetti 2628 gagggcattt atgtgattta gaacttgatt amtgtttcac tgtgtatgtt caacactacc 2688 tgtaatatit taactaaagc tatttaatgt aatatgatgt gtatacattc tgtaaattaa 2748 tttttaaato totasstago tttaagttgo tatogtgsta tttottttad aastcamaat 2808 aaatottttt ggaatgataa taasaaaaa aasaaa 2844

<210> 4

<211> 495

<212> PRT

<213 > Ovis ap.

c4005 4

- Met Lys Ser Pro Val Ser Asp Leu Ala Pro Ser Asp Gly Glu Glu Gly 1 5 15
- Ser Asp Arg Thr Pro Leu Leu Gln Arg Ala Pro Arg Ala Glu Pro Ala 20 25 30
- Pro Val Cys Cys Ser Ala Arg Tyr Asn Leu Ala Pho Leu Ser Phe Phe 35 40 45
- Gly Phe Phe Val Leu Tyr Ser Leu Arg Val Asn Leu Ser Val Ala Leu 50 55 60
- Val Asp Net Val Asp Ser Asn Thr Thr Ala Lyo Asp Asn Arg Thr Ser 65 70 75 80
- Tyr Glu Cys Ala Glu His Ser Ala Pro Ile Lys Val Lau His Asn Gln 85 90 95
- Thr Gly Lys Lys Tyr Arg Trp Aep Ala Glu Thr Gln Gly Trp Ile Leu 100 105 110
- Gly Ser Phe Phe Tyr Gly Tyr Ile Ile Thr Gln Ile Pro Gly Gly Tyr 115 120 125
- Val Ala Ser Arg Ser Gly Gly Lys Leu Leu Gly Phe Gly Ile Phe 130 135 140
- Ala Thr Ala Ile Phe Thr Leu Phe Thr Pro Leu Ala Ala Asp Phe Gly
  145 150 155 160
- Val Gly Ala Leu Val Ala Leu Arg Ala Leu Glu Gly Leu Gly Qlu Gly
  165 170 175
- Val Thr Tyr Pro Ala Met His Ala Met Trp Ser Ser Trp Ala Pro Pro 180 185 190
- Lau Glu Arg Ser Lys Leu Leu Ser Ile Ser Tyr Ale Gly Ala Gln Lau 195 200 205
- Gly Thr Val Val Ser Leu Pro Leu Ser Gly Val Ile Cys Tyr Tyr Met 210 215 220
- Asn Trp Thr Tyr Val Phe Tyr Phe Phe Gly Ile Val Gly Ile Ile Trp 225 230 235 240
- Phe Ile Leu Trp Ile Cys Leu Val Ser Asp Thr Pro Glu Thr His Lys
  245 250 255

Thr lle Thr Pro Tyr Olu Lys Glu Tyr lle Leu Ser Ser Leu Lys Asn 260 265 270

- Gln Leu Ser Ser Gln Lya Ser Val Pro Trp Ile Pro Met Leu Lya Ser 275 280 285
- Leu Pro Leu Trp Ala Ile Val Val Ala His Phe Ser Tyr Asn Trp Thr 290 295 300
- Phe Tyr Thr Leu Leu Thr Leu Leu Pro Thr Tyr Met Lye Glu Val Leu 310 315 320
- Arg Phe Asn Ile Glm Glm Asn Gly Phe Leu Ser Ala Val Pro Tyr Leu 325 330 335
- Gly Cys Trp Leu Cys Met Ile Leu Ser Gly Gln Ala Asp Asn Leu 340 345 350
- Arg Ala arg Trp Aen Phe Ser Thr Leu Trp Val Arg Arg Val Phe Ser 355 360 365
- Leu Ile Gly Met Ile Gly Pro Ala Ile Phe Leu Val Ala Ala Gly Phe 370 375 380
- Ile Gly Cys Asp Tyr Ser Leu Ale Val Ale Phe Leu Thr Ile Ser Thr 385 390 395 400
- Thr Leu Gly Gly Phs Cys Ser Ser Gly Phs Ser Ile Asn His Leu Asp 405 410 415
- Ile Ala Pro Ser Tyr Ala Gly Ile Leu Leu Gly Ile Thr Asn Thr Phs 420 425 430
- Als Thr Ile Pro Gly Met Ile Gly Pro Ile Ile Ala Arg Ser Leu Thr 435 440 445
- Pro Glu Asn Thr Ils Gly Glu Trp Gln Thr Val Phe Cys Ile Ala Ala 450 455 460
- Ala Ile Amn Val Phe Gly Ala Ile Phe Phe Thr Leu Phe Ala Lya Gly 465 475 480
- Glu Val Gln Asn Trp Ala Ile Ser Asp His Gln Gly His Arg Asn 485 490 495

4210> 5

<211> 31

<212> DNA

<213> Artificial Sequence

<223> Description of Artificial Sequence: PCR primer

egggetocog congenatge ayrabristg g

31

<210> 6

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer

<400> 6

ggaattccdg gdgcratktc narrtrrtt

29

<210> 7

<211> 2930

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (263)..(1870)

<400> 7

gttoggtoga agecetecco ttaattatgt gcastteaag teeccactge cogecegoaa 60

geccccacte attetegety egggcagggt ggcccctgca ctttacaagg gggtgcagga 120

gogggagaeg gtogteogaa caeeggeteo coggeatgeg tagacoggeg ggoggagogg 180

geteactity egecmatect aegagaacte ecagametre getteectag teosacceaa 240

gecagagetg eccacaceta ag atg geg geg geg atg aca ceg ecc ege 292 Met Ala Ala Gly Ala Met Thr Pro Pro Arg

10

ceg gte cag con get egg coc ggg gge tte ggg etg trg gge egg ege Pro Val Gln Pro Ala Arg Pro Gly Gly Phe Gly Leu Ser Gly Arg Arg 15

r.	<b>C C t</b>		CC E	.gc	cag	gtg	- <b>\$</b> C	g aç	t a	ca d	ct	get	C da	80 6	tta	ana	- ^-	+ -	atg	200
Se	r Le	u L	eu C	ys I	Gln	Val	Ala	a Se	r T	hr s	ero.	Ala	• Hi	i	1-1	33	· 9	- 1	Met	388
			٠	30						35	·		~	•	31			4 T	Mec	
									,							4.0	)			
ACT	n to	et or	ים מ		^~~															
h =	- c.		~9 9 •= 11	-7 1	-ya	940	CLE	i de	c ci	39 a	ac	gat	99	ic ā	æg	gag	a g	jc	acg	436
vī	9 26	r Pi	70 V	91 1	arg	qaA	Let	1 AL	a Ai	rg A	ទីវា	Yat	Gì	y G	JЛ	Glu	: Se	r	Thr	
		•	15					5	0			•			55					
ga	c cg	C 40	â c	et c	tt	ctà	ceg	99	c go	e e	¢a	caa			aa	d'è-		•	~~·	40.
Aej	P Ar	g Tì	IF P	ro I	eu :	Leu	Pro	01	עבֿ ע	a 2	ro	Aro	D)	- E	111	37-	*1	_	D	484
	б	<b>O</b>					65			_				0	- 4	WIG	WI		PIO	
													,	·						
ato	ı ta	ė to	e Fi	et e	iae i	cat.	<b>F</b> = A	4	- +÷											
Va 1	~~ ~~	e tg	, e		1.		Dag.	Bet.		n G	Cal	act	CL	3 34	cc '	ttt	tt	t :	<del>gg</del> t	532
75	. ~,	в Су	<i>a</i>	EL W	45 /	Kr <del>y</del>	lyr	Agi	ı Le	u A	18	I10	Lei	L A.	la	Phe	Ph	ė	Gly	
7.2	•					80						85							90	
Etc	tt	e at	t gt	g t	at ç	(Ca	tta	¢gt	gt:	g 48	it (	ctg	agt	gt	:t <	aca.	tt	9. 6	sto	580
Phe	Ph	11	e Va	1 T	yr A	lla.	ùeu	Arg	Va.	l As	ו תו	Leu	g e z	. Vs	a i	A]a	7.61	- ;	7-3 7-1	200
				:	95					10					•		10		741	
																	+0:	•		
gat	etg	gt	A ga	t to	ca a	at.	aca	Bož	. bbs	. <u> </u>										
Aep	Net	Va	l As	n 86	 4 4e	971	The	The	Las	, 61			2		<b>a</b> . a	ICT	-	3 8	ag	628
•			11	<u>г</u>				****			u. P.	яр	WOL	Ar			Sei	·	·ys	
				•					115	•					1	20				
~~~	+																			
373	dy.	CCE	. 9a	9 6		Ct (366	CEC	acı	34	a g	tt	cat	CE	t a	æt	C&A		cg	676
Wrg	Cys	Pro) GT.	u Hi	.a Ş	er ;	Ala	PTO	Ile	Ly	вγ	al	Hie	Нi	ø A	en:	Gln	T	hr	
		125	•					130						13	5					
															•					
ggt	sså	489	, ta	C CO	ua tç	9 9 9	jet .	Ģ⊄z	gaa	açi	tc	aa I	gga	tg	; a	tt .	cte	•	σt	724
Gly	Lys	Lys	Ту	r G 1	n Ti	rp J	gs.	Ala	Glu	Th	r G	ln (01 v	Tri	- D I.	le i	Leiu	- C	⊒- ไบ	
	140						.45						150	1	-	-+ .		_	-,	
																	٠			
tcc	ttt	ttt	tat		c ta	ıć a	te /	at c	Ara	C 97		-+	+							
Ser	Phe	Phe	TVI	- G1	u Th	 	۱۵ ۰		The	214			GU L	994	9	ga. 1	ac.	a.	E T.	772
155			-,-		, ., 16		16 ,		1ДГ.	GT1			PTO	617	4 G .	Ly 1	Υ.			
					7.0	,,,					11	55						1:	70	
	~																			
966 31-	aye	Baa	ata	399	99	g a	43 8	ıtg	ctg	cta	35	a t	tt	99 9	at	ie c	tt	82	ÇĊ	3 20
ALA	ger	Lya	Ile	913	y al	УL	ys þ	ist	Leu	Lev	4	ly i	?he	Oly	11	le I	eu	G1	ly	
				175						180							.85		-	
act	gcŁ	gtc	Ctc	BÇÇ	ct:	g ti	tc a	ct	200	att	ac	et a	rca	gat	ŧ.	a r	פש	a+	•	868
rat T	Ala	Val	Leu	The	. Le	u Pi	ne T	hr	Pro	Ile	ت 1 <u>ا</u>	- 3 - 1)e	3	1.0	- 3	1	7/~	1	900
			190				-		195				4	باه			- y	Υđ		
									-73						20	Ų				
102	CC2	cto		~- -		.			2 .									•		
77 T	D2~	ctc	T?	45-1	. UL	- 20	19	달라 (기 _		gar	99	a c	ce	389	89	9 9	gt	gt	t	916
7	6 T C	ren	774	AST	. #61	u Ai			Leu	Glu	Gl	y L			Gl	и G .	ly '	٧a	1	
		205					2	10						215						

aca	Ett	; ⊂ ¢4	, ac	e at	g ca	t go	c at	g tg	g cc	t tc	t tg	g qc	E &C	0	=t	ctt	964
Thr	Phe	Pro	Ala	a Me	t Hi	s Al	a Me	t Tr	p Se	r Se	r Tr	ובים	. Dr	n Di	-	T = 15	
	220					32					23					Dec	
	V	,					-				23	V	•				
	-																
gaa	Aga	ı ago	S.B.B.	l Ct	t ct	t ag	c at	t to	g ta	t gc	å 9 9	a gç	\$ ¢a	g et	it g	39 9	1012
Glu	Arg	, Se	Lys	Le	u Le	u 3e	r Il	e Se	נע?	r Ala	a Gl	v A).	a Gî	_ n 1.4		21 v	
235					24				,	245		,		44			
						_				47.	,				•	2.5 D	
aca	gca	att	CCC	CE!		tct	t tct	gg.	356	ı att	: tg	c ta	ta ta	t aç	9 4	at	1060
Thr	Val	Ile	Set	Lei	ı Pr	o Fe.	u Sei	Gi)	y Ile	: Ile	E Cyl	з Туг	t Ty:	r Me	t A	nej	
				259			-		260					26			
															_		
tea	act	Eat	ata	++-	+ +=	• ttl	ttt	ەندىم ،									
733 EEC-	mb-	~-	11-1	25-			- Dh	39,	- 4500		99.	. aca	5 CC1	tg	9 t	.EE	1108
тър	1111	yyı			YY1	. Rue	Phe	; GT)	Thi	Ile	GI	/ Ile	Ph:	TI	P E	he	
			270	1				275	5				280)			
					•			,									
ctt	ttg	tgg	ato	tgg	tta	qt	agt	Gác	aca	cea	CAS	1.3	ra/				1166
Leu	Leu	Ŷrd	Tla	Tro	l Len	Val	. Ser	. Dan	The	Dwa	01-		. ud.			2º	1156
		285							. 1411	FIU	. 441			Ly	8 A	rg	
		483					290					295	i				
									•								
att	tcc	CAC	tet	gae	Aag	gaa	tac	att	ctt	tca	tca	Lta	Aga	aat	t c	a g	1204
Ile	Ser	His	Tyr	Glu	Lya	01 u	Tyr	Tle	Leu	8er	Ser	Leu	Arq	Agı	a 0	ln.	
	300					305					310		3		_		
A++	++	+47						.									
• • • •	2	Con	cay	aay		949	_ C¢g	L99	gta	C	acc	tta	ABA	CCC	; c	tg	1252
_	ser	ser	Gin	гÃв	Şer	VAI	Pro	Trp	Val	Pro	Ile	Leu	Ly4	801	L	LU	
315					320					325					33	3 D	
		•															
CCB	ctt	tgg	gct	ate	gta	gtt	gca	cac	t±t	tet	tac	880	taa	act		. 4	1300
Pro	Leu	TEN	Ala	Ila	Va 1	1/21	Ala	Wie	ōho.	Par	The	2	-23 	mb			1300
				335				****		SCT	117	HBIL	TIP			14	
				77.7					340					345	•		
tat	act	tta	ttg	BÇA	tta	ttg	CCE	act	tat	Atg	zag	gag	atc	cta	ag	M	1346
Tyr	Thr	Leu	Leu	Thr	Lau	Leu	Pro	Thr	Tyt	Met	Lys	Glu	I1e	Lau	AT	•	
			350					355	•		•		360			7	
													300				
	441	get	CHA	949	AAC	999	ttt	LEA	CCL	cca	ttg	cct	tet	tta	88	C	2396
Phe	Asn	Val	Glu	Glu	Asn	gly	Phe	Leu	Ser	Ser	Leu	Dto.	Tyr	Leu	Gl	Y	
		365					370					375					
tet	taa	tta	tat	ate	ate	උදුල	tet	au.	79.	444	أطمين	M > 7	A D =	b 4 -		_	1444
																	1444
		∠ ou	-78	m e L	116		Ser	GTÅ	ATD			A6P	ASI	PBA	AZ	9	
	380					385					390						
jca .	a a a	tgg	aat	ttt	tca	act	tta	tgt	gtt	CQC	aga	att	ttt	a 90	Ct.	t	1492
Ma:	Lys	Trp	Ren	Phe	Ser	Thr	Leu	Çva	Val	- Arva	- Ara	Ilė	Phe	SPT			
95	- '	-			400			- / -		4D5		D	~ 1140	1	81		-
										- U 2					B 7 1		

aLa	299	7 765	, ACC	934	cet	gca	1 865	, tto	: ¢tg	j gta	a geo	get	990	tte	act	1540
Ile	Gly	Met	: Ile	Gly	Pro	Ala	. Val	Phe	: Leu	. Vai	Ala	. Ala	Gli	/ Phe	: Ile	1340
				435					420					425		
														442	•	
000	F .4+	- + F	*	F-04	* * *											
23-	cyc	940		CUL	rt Ş	acc	acr	. acr	ECC	cta	act	. ata	t ¢a	aca	aca	1568
ery	CAB	жер			Leu	Ala	Val	Ala	Phe	Leu	The	Ile	Ser	The	Thr	
			430					435					440			
ctg	gga	ggc	ttt	t ga	tet	tct	qqa	ttt	age	atc	aac	cat	C# fr	mat		202
Leu	Glv	giv	Phe	CVS	Ser	Ser	สาร	Dhe	242	11-	hon	210	1	Sar	#CC	1636
		445		-, -		•••	450				72011		T-E (I	ден	119	
							. 430					455				
		4		٠.												
gec	-CF	rcs	tat	act	ggt	aţç	ctc	ctg	ggc	atc	aca	aat	\$¢a	ttt	₫ ¢≎	1684
TTØ	Pro	Ser	Tyx	ala	Gly	Ιlα	Leu	Leu	Gly	Ile	Thr	Asn	Thr	Phe	Ala	
	460					465					470					
act	att	cca	gga	atq	att	gaa	éée	gtc	att	act	222	AOF	cta	200		
Thr	Ila	Pro	Gly	Met	Val	លាប	PTO	Val.	714	n?-	tua	CAM			CCT	1732
475						4.,	-10	741	114		пуa	oet	Ten	TUX		
4.5					480					485					490	
gac	aac	açt	gtt	33 9	gaa	tgg.	CAA	acc	gtg	ttc	tat	att	gct	get	get	1780
qaA	Ast	Thr	Val	Gly	Glu	Trp	Gln	The	Val	Phe	Tyr	Ila	BÍÁ	Ala	Ala	
				495	٠,				500					505		
att	aat	qtt	ttt	aat	acc	att	tte	ttt	aca	cta	ttc	ace				3000
Ila	Aan	Val	Phe	Glv	11a	f) a	Dhe		The se	Lou	Dh.	314	***	234	à-r-r	1829
			510	,		-14		515		пеп	ьпф			GIA	GIR	
•			310					313					520			
			_													
			tgg													1870
VAl	Gin	Asn	Trp .	ALA :	Leu .	Vels '	Arp	His .	Hie	GJA	Kia	Arg i	H[s			•
		525					53 à					535				
tgaa	9988	cc a	ataa	ataa	t cei	tacal	tcta	tta	.tata	att	et e a i	thtai	te a	177 + 4	a ccte	1 624
						-							-	-3		1330
aaaa'	tocc	tt e	tätai	re-me	T + B.			atori	- ~++1						teega	
	-3		-3 tm	- 43 4	3	-3-4-01		a 031			LCdd	rrasi	#C C	cars	CERGA	1990
C & C C	cesă	ac c	cacaa	atcai	c gaa	teje	CBC	cagi	tgc	cag (BB T A !	read:	ea t	388¢	tgtgt	2050
tteat	ttat	ga a'	taati	itgti	age A	tagg	jact	tota	cttt	Ag s	at to:	cate	ic ci	igac:	tgcta	2110
															-	
rteg	gca	ac al	tgaac	rtago	aca	atto	:tat	toat	ttet	ta e	18000	eatar	et as	1900	jiatg	7176
	- 🕶 "			, 31	,		- 3 -	-3-4		1	335¢			37	aecg.	Z1 /Q
	7300		- 4 54 164 1	·*					.					_		
~y ~ L!	90056	re gi	-6256	erga	CAC	CEEC	gec	Cast	CRAA	ICT 8	ıgatç	 4658	t to	tcag	gtac	2230
gatı	laac	8C C	gttg	ttgt	tca	cttt	cct	cata	8465	tt g	rtcag	jctct	c to	tgac	actt	2290
													٠			
gaci	:tca/	a ct	ttao	cato	tet	a t.aa	auc	tacc	atec	ac t	ote t	Aath	+ ~			2250

ctggactgag gggagtggag ccaaggaget gecaaggact centecetg ctteagggte 2410
agagtgeera gegtttatea gaggaggat ccaaggaccag agec gtgte gactettegg 2470
ctggtgeert teetetgagg ggetateaat gtgtagataa agecetgagt aggaaagag 2530
agtgagatee actgetatgg tettgataca teeteaaget tteeetteee ageacagagg 2590
aatattgget ggeatgaac etggaaaaga aaaataggaa geggeeggge aeggtggete 2630
atggeetgtaa teecageact ttgggggget gaggtgggeg aateatgaga teaggagtte 2710
gagaccagee tggeeagat ggtgaaacee eatetetaet aaaaatacaa aaaataget 2770
gggegtggtg aegggeget gtaateecag atacteagga ggetgaggta ggagaateac 2830
ttgaacctgg gagattgaag ttgcagtgaa ccaagatcac gecactgeac teeagcetgg 2890
gegatggage gagattecaa eteaaaaaa aaaaaaaaa

<210> 8

<211> 536

<212> PRT

<213> Nomo sapiens

<400> 8

Met Ala Ala Cly Ala Met Thr Pro Pro Arg Pro Val Gln Pro Ala Arg 1 5 10 15

Pro Gly Gly Phe Gly Leu Ser Gly Arg Arg Ser Leu Leu Cys Glm Val 20 25 30

Ala Ser Thr Pro Ala His Val Gly Val Met Arg Ser Pro Val Arg Asp 35 40 45

Leu Ala Arg Asn Asp Gly Glu Glu Ser Thr Asp Arg Thr Pro Leu Leu 50 55 60

Pro Gly Ala Pro Arg Ala Glu Ala Ala Pro Val Cys Cys Ser Ala Arg 65 70 75 80

Tyr Ash Leu Ala Ile Leu Ala Phe Phe Gly Phe Phe Ile Val Tyr Ala 85 90 95

Leu Arg Val Asn Leu Ser Val Ala Leu Val Asp Mat Val Asp Ser Asn 100 105 110

- Thr Thr Leu Glu Asp Asn Arg Thr Ser Lys Ala Cys Pro Glu His Ser 115 120 125
- Ala Pro île Lye Val Lie His Ach Gln Thr Gly Lys Lye Tyr Gln Trp 130 . 135 140
- Asp Ala Glu Thr Gln Gly Trp Ile Leu Gly Ser Phe Phe Tyr Gly Tyr 145 150 155 160
- fle He Thr Gln He Pro Gly Gly Tyr Val Ala Ser Lys He Gly Gly
 165 170 175
- Lys Net Leu Cly Phe Gly Ils Leu Gly Thr Ala Val Leu Thr Leu 180 185 190
- Phe Thr Pro Ile Ala Ala Asp Leu Gly Val Gly Pro Leu Ile Val Leu 195 200 205
- Arg Ala Leu Glu Gly Leu Gly Glu Gly Val Thr Phe Pro Ala Met His 210 225 220
- Ala Met Trp Ser Ser Trp Ala Pro Pro Leu Glu Arg Ser Lys Leu Leu 225 230 235 240
- Ser Ile Ser Tyr Ala Gly Ala Gln Leu Gly Thr Val Ile Ser Leu Pro 245 250 255
- Lau Ser Gly Ile Ile Cys Tyr Tyr Met Asn Trp Thr Tyr Val Phe Tyr 250 265 270
- Phe Phe Gly Thr Ile Gly Ile Phe Trp Phe Leu leu Trp Ile Trp Leu 275 280 285
- Val Ser Asp Thr Pro Gln Lys His Lys Arg Ile Ser His Tyr Glu Lys 290 295 300
- Glu Tyr Ile Leu Ser Ser Leu Arg Asn Gln Leu Ser Ser Gln Lys Ser 305 310 315 320
- Val Pro Trp Val Pro Ile Leu Lys Ser Leu Pro Leu Trp Ala Ile Val 325 330 335
- Val Ala His Phe Ser Tyr Asn Trp Thr Phe Tyr Thr Leu Leu Thr Leu 340 345 350
- Leu Pro Thr Tyr Met Lys Glu Ila Leu Arg Phe Asn Val Glu Asn 355 360 365

Gly Phe Leu Ser Ser Leu Pro Tyr Leu Gly Ser Trp Leu Cya Met 11e 370 375 380

Leu Ser Gly Gln Ala Ala Asp Asn Leu Arg Ala Lys Trp Asn Phe Ser 385 390 395 400

The Leu Cys Val Arg Arg Ile Phe Ser Leu Ile Gly Met Ile Gly Pro

Ala Val Phe Leu Val Ala Ala Gly Phe Ile Gly Cys Asp Tyr Ser Leu 420 425 430

Ala Val Ala Phe Leu Thr Ile Ser Thr Thr Leu Gly Gly Phe Cys Ser 435 440 445

Ser Gly Phe Ser Ile Asn His Leu Asp Ile Ala Pro Ser Tyr Ala Gly
450 455 460

The Leu Leu Gly The Thr Asn Thr Phe Als Thr The Pro Gly Met Val

Gly Pro Val Ile Ala Lys Ser Leu Thr Pro Rap Asn Thr Val Gly Glu
485 490 495

Trp Gln Thr Val Phe Tyr Lie Ala Ala Ala Lle Asn Val Phe Gly Ala
500 505 \$10

Ile Phe Phe Thr Leu Phe Ala Lys Gly Glu Val Glm Asn Trp Ala Leu
515 520 525

Asn Asp His His Gly His Arg His 530 535

<210> 9

<211> 1485

<212> DNA

<213> Artificial Sequence

c220>

<223> Description of Artificial Sequence: human/sheep consensus sequence

<220>

<221> CD6

<222> (1)..(1485)

<40	D> 9	1														
															grgc	48
Met	Xaa	: Xaa	Pro	Val	. Xaa	ABP) Xaa	ı Ala	хаа	Xa:	а Хаа	Gly	Glo	Gli	ı Xaa	
1				5	.				10)				1	5	
	-															
weg	gac	cgo	acr	cck	cty	, cti	cmg	вдс	get		ර එමුල	gcs	gas	S C C	gat	96
															Ala	
			20					2.5			_		30			
CCB	gtr	tgc	tge	tet	gct	cgt	tac	aac	yta	gca	wtt	ttg	kac	ttt	ttt	144
											Xaa					
		35				-	40					45				
ggt	ttc	tte	rtt	ete	tat	kca	tta	cgk	gtg	aat	ctg	4 97	act	чсі	vta	192
											Leu					132
-	50				•	55					60				********	
gtg	gay	atg	gtr	gat	tca	aay	aca	act	kym	raa	gat	sat	aga	ack	tee	240
											Asp					
65				•	70					75	_		3		80	
															••	
YA.S	gmg	tgt	aca	geg	cat	tct	get	466	ata	aaa	gtt	cwt	CBV	187	CAR	288
											Val					7**
		•		85					90	-		•		95		
		•														
acg	ggt	aar	aag	Các	CII.	tgg	gat	gça	gaa	act	CAR	प्रप्रव	८व्रप	att	ctc	336
											3ln					
	_		100	_		-	-	105				•	110			
MEE	tey	ttt	tty	tat	ggc	tac	ate	atc	aca	CBT	att	cct	gga.	998	tat	384
											Ile					
		115					120		•			125	•	•	•	
gtt	gee	agç	ATA	akw	999	999	aax	mtg	ytg	cta	gga	tty	999	atç	ytt	432
											Gly					
	130					135					140		-			
gsy	ACW	gct	rto	ytc	acc	ctg	tte	act	ccc	nty	gct	gca	gat	ttm	qqa	480
-										_	Ala	_	_			
145					150					155			_		160	
gty	gga	scm	cty	rtŁ	gya	ctc	agr	gca	Cta	gaa	ggr	cta	gga	gag	ggt	526
											Xaa					
	•			165					170			-	-	175		
gty	aca	twt	CCA	gee	atg	cat	gca .	ato	tga	tct	tcw	Ega -	act	ccc	ect.	576
		Xaa										Trp	-			
			180					185	•-			_	190	- 		

Ct	c ga	a ag	a ag	1C 36	ar ct	ני כו	tk as	gy at	tt to	er t	at	gca	998	a ge	a c	ar	ctt	62
Lei	π Ĉĵ		g Se	Y Xa	ia Le	eu X4	a k	aa I	le Xa	aa T	γr	A) #	Gly	r Al	a X	ae	Leu	
		19	5				20	00					205	5				
	-																	
999	g ac	a gt	a rt	t to	t ct	t co	t ct	t to	t gg	a r	¢a i	att	tgo	te:	c t	at	atq	67
Gly	/ Th	r Va	1 Xa	a 9e	r Le	n Pr	o Le	au Se	r G	y x	aa :	lle	Cys	Ту	T T	yr	Met	
	21					21						220	-	•		•		
aat	: tg	g ac	t ta	t gt	c tt	c ta	y tt	y tt	८ पुत्र	y ay	/¢	ctt	QQ8	at	m wri	υ	ten	720
Ast	Teg	מד	r Ty	r Va) Ph	e Xa	a Xa	a Ph	e Xa	A X	la)	(aa	Gly	Xa	a Xe		Tra	, ,
225	i				23					23			-		•		340	•
ttt	mtt	tt	r tgg	g at	c tg.	s tt	a ot	tag	t ga	V AC	a c	ca	BBB	Ams	af 178			200
Phe	Xaa	ı Xa	a Try	- - 11	e Xa	a Le	- J- ц Va	l Se	r Xa	• Th	ir P	מתו	Xaa	Ya:	· π4	_	a a y	766
				24					25					12.21	- 112 25		чур	
										-					*3	-		
968	aty	r was	y cmy	/ tal	t da:	1 220	σa:	r ta	v ati	t et	tt	C.a.	tce	 -				
Xaa	Xaa	Xaa	Xae	L Tv:	r Oli	i Lvi	B Xa	a Xe.	, Il.	L	u ŝ	27	CAH	Las	. ve		aat 	816
			260			,.	_ ,_,_,	26.			• •		-4.	270		A.	ABI	
														۵/۱	,			
cag	cty	tet	tca	cac	T ARC	r bes	a arta		ı tac	r et.	8 C	av :	4+b	. ete e		_		
Gin	Xaa	Ser	Ser	Gle	l lve	80	Val	Dr.	7 - 22	y Ye.	n Y	-1	Hun. Yas	Art	- Arci	d.	두다	864
		275			3 1		286				- 41		285	ARG	· Ly	ь,	A B B	
												•	603					
ctg	CCB	ctt	tgg	dat	atv	ort.n	nentr		-	, ++1	- +,	-t (.		
Leu	Pro	Leu	Tap	Ala	Xaa	Xee	. Val	nie Lie	Yas	Dina			The	No.	rāi	3 4	FCE	912
	390					295						=r . 30	ιγε	WRII	121	9	CELE	
							•				31							
ttt	tat	act	ttr	Lta	6.00	Ltta	- Eto	cet	act	F 4 9		٠		×				
Phe	Tyr	Thr	Xaa	Leu	Yaa	Leti	Levi	משם ו	The	Yes	r. W.L		rag	Act	¥		AJE	960
305	- , -			-	310					315		9 L	n), R	Y64	Yge			
					010					313	'					3	20	
AQC	tte	aat.	rtt	CRA	ana		-	+++	***	+-+	1	1.	· -				_	
			Xaa															1008
				325	700		OL y	F 1263	330	ael	A.Q.		adici.	5.EQ	•		æu	
									330						335	•		
actv	tat	toor	tta	tat	eta	p to	at a	مامده	~~+	A	~=						_	_
																		1056
-		111	Leu 340	Cyn	MEL	TTA	rac	345	GIA	LIII	44-7-	A A			APN	L	ev	
			310					740					4	350				
900	~~-								44 _ 1.		_							
			tgg															1104
J	~10		Trp	WST;	FMS	per		vas	YAB	AST	X#4			Lan	Phe	8	er	
		355	•				360					. 30	55					
~+ +															•			
			acg															1152
	420 TT6	aab.	Met	YTE	GTÅ	P70	ae.	ABA	rne	₽¢#			aa X	AA	ZZZ	X	0.	
	4 J N					276					207							

atw	99c	tgt	gat	tat	tcy	ttg	aca	gtt	. acm	ttc	ote	acy	ats	tca	aça	1200
Xaa	Gly	CAB	Aep	Tyr	Хжа	Leu	Xaa	Val	Xaa	Phe	Lat	Xae	116	Ser	Thr	
385					390					399	;				400	
acm	ctg	gga	990	ttt	tg¢	tct	tot	99&	ttt	ago	ato	aac	cat	ctg	gay	1248
XBA	ֆբu	Gly	Gly	Phe	Cys	Ser	Ser	Gly	Phe	Ser	īle	Asn	His	Leu	Хаа	
				405					410					415		
att	gct	çct	tcg	tat	ğet	ggt	aty	ctc	ctg	ध्रप्र⊂	ato	aca	aa¢	0.¢π	ttt	1296
Ile	Ala	Pro	Ser	Tyr	Ala	Gly	Xas	Leu	Leu	Gly	Ile	Thr	Asn	Xaa	Phe	
			420			•		425		•			430			
gcc	act	att	ÇÇW	gga	atg	rtt	999	ccc	rtc	att	ясу	ara	agt	ctk	acr	1344
														Xaa		
		435		-			440					445			-110	
cct	gak	23 C	act	rtt	gga	gaa	tgg	Cad	acy	gtik	ttc	try	atv	get	act	1392
														Ala		
	450				_	455	•				460					
											•					
get	aty	ast	gtw	ttt	ggt	ġcc	att	ttc	tty	ACA	cta	ttc	gcc	888	aat.	1440
Ala	XAA	Asn	XAA	Phe	dly	Ala	Ile	Phe	Xaa	The	Lau	Phe	Ala	Lys	Glv	
465			-		470					475					480	
gaa	gtr	caa	aac	tgg	acy.	mte	AFC	gat	CAC	OBW	455	cac	aga	TIAC		1485
Glu																2000
				485				_	490		•			495		

<210> 10 <211> 495 <212> PRT <213> Artificial Sequence

<400> 10

Met Xaa Xaa Pro Val Xaa Asp Xaa Ala Xaa Xaa Xaa Gly Glu Glu Xaa 2 5 10 15

Xaa Asp Arg Xaa Xaa Xaa Xaa Xaa Ala Xaa Arg Xaa Glu Xaa Ala 20 25 30

Pro Xaa Cys Cys Ser Ala Arg Tyr Asn Xaa Ala Xaa Leu Xaa Phe Phe 35 40 45

Gly Phe Phe Xaa Xaa Tyr Xaa Leu Xaa Val Asn Leu Xaa Val Xaa Xaa 50 55 60

Val Xas Met Xas Asp Ser Xas Thr Thr Xas Xas Asp Asn Arg Xas Ser
65 70 75 90

- Xaa Xaa Cys Xaa Glu His Ser Ala Pro Ile Lys Val Xaa Xaa Xaa Gln 85 90 95
- Thr Gly Xas Lys Tyr Xas Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu 100 105 110
- Xaa Xaa Phe Xaa Tyr Gly Tyr Ile Ile Thr Xaa Ile Pro Gly Gly Tyr 125 120 125
- Val Ale Ser Xae Xae Gly Gly Xae Xae Leu Gly Xae Gly Ile Xae 130 135 140
- Kaa Xaa Ala Xaa Xaa Thr Leu Phe Thr Pro Xaa Ala Ala Asp Xaa Gly
 165 150 155 160
- Xaa Gly Xaa Xaa Xaa Xaa Leu Xaa Ala Leu Glu Xaa Leu Gly Glu Gly 165 170 175
- X88 Thr Xas Pro Ala Met Ris Ala Met Trp Ser Xas Trp Ala Pro Pro 180 185 190
- Leu Glu Arg Ser Xas Leu Xas Xas Ile Xas Tyr Ala Gly Ala Xas Leu 195 200 205
- Gly Thr Val Xma Ser Leu Pro Leu Ser Gly Xma Tle Cys Tyr Tyr Met 210 215 220
- Asn Trp Thr Tyr Val Phe Xaa Kaa Phe Xaa Xaa Xaa Gly Xaa Xaa Trp 225 230 235 240
- Phe Xaa Xaa Trp Ile Xaa Leu Val Ser Xaa Thr Pro Xaa Xaa His Lye 245 250 255
- Xaa Xaa Xaa Xaa Tyr Glu Lys Xaa Xaa Ile Leu Ser Ser Leu Xaa Asn 260 265 270
- Gln Xaa Ser Ser Gln Lys Ser Val Pro Trp Xaa Xaa Xaa Xaa Lys Xaa 275 280 285
- Leu Pro Leu Trp Ala Kas Kas Val Ala Kas Phe Ser Tyr Asn Trp Thr 290 295 300
- Phe Tyr Thr Xaa Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xaa Leu 305 310 315 320

Arg Pho Asn Xaa Gin Glu Asn Gly Pho Leu Ser Xaa Xaa Pro Tyr Leu 325 330 335

Xaa Xaa Trp Leu Cys Met Ile Leu Xaa Gly Gln Ala Als Asp Asn Leu - 340 345 350

Arg Ala Xaa Trp Asn Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser 365 360 365

Xaa Gly Cys Asp Tyr Xaa Leu Xaa Val Xaa Phe Leu Xaa Ile Sar Thr 385 390 395 400

Xaa Leu Gly Gly Phe Cys Ser Ser Gly Phe Ser Ile Asn His Leu Xaa 405 410 415

Ile Ala Pro Ser Tyr Ala Gly Xea Leu Leu Gly Ile Thr Asn Xea Phe 420 425 430

Ala Thr Ile Xaa Gly Met Xaa Gly Pro Xaa Ile Xaa Xaa Ser Xaa Thr 435 440 445

Pro Xaa Asn Thr Xaa Gly Glu Trp Gln Xaa Xaa Phe Xaa Xaa Ala Ala 450 455 460

Ala Kaa Asn Xaa Phe Gly Ala Ile Phe Xaa Thr Leu Phe Ala Lys Gly

Glu Xaa Gln Asn Trp Xae Xaa Kaa Asp His Xaa Gly His Arg Xae 485 490 495

<210> 11

<211> 1485

<212> DKA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: human/sheep consencus sequence

<220>

<221> CD8

<222> (1).,(1485)

<400> 11

9 t g	g ditte	g tc	ה ככ	9 90	t m	on da	C DF	n gc	c cr	8 41	uc de	an gg	ge g	ag ç	jag	ngo	4 4
Mat	. Xa	a Xe	a Pr	o Va	il Xa	eA s	р Ха	a Al	a Xa	a Xa	ex sa	a G	ly G	lu (31 u	Xaa	:
1	L				5					0			-		15		
			•														
ಬರದ	ga.	c cg	c ac	u cc	n et	n et	n eng	ng:	e ge	c c c	m cg	ig go	ற ஒ	aa 1	ıce	qct	96
Xaa	ı Asj	rA q	g Xa	a Xa	a Xa	a Xa	e Kaa	a Xa	a Al	a Xa	ra Ar	y Xe	A G	lu x	(aa	Ala	
			2					2:				-		30			•
													•				
CC6	gt	n tg	c tg	c tc	t gc	t cgi	t tac	2 866	nt.	a gç	a nt	t Et	a no	-e t	t.t.	+++	14
Pro	Xas	Çy:	в Су:	s Şe	r Al	a Arg	Tyx	. Ası	Xa	a Al	a Xa	a Le	u Xa	LA F	he	Phe	7-7
		3					40						5			- 40	
ggt	tte	tt.	nti	t nt	n ta	t nc	tta	cgr	gte	3 aa	t ct	T AG	n et	t a	сn	nte	19
Gly	Phe	Pho	XA:	a Xa	а Ту	r Xaa	Leu	Kaa	Va	l AB	n Lei	u Xa	a Va	ıl x	A.A	Yas	
	50)				55					61		- '-			nea	
gtg	gan	atg	gti	ga:	t te	a aan	aca	act	nor	na.	a gat	. 88	t ao	a a	СIJ	tee	24
Val	Xaa	Met	: Xas	رعد	se:	r Xaa	Thr	Thr	Xaa	ı Xa	s Ast	AS C	n Ar	or X.	n A	Ser	64
65					70						•			J		80	
											•					•	
nan	gng	tgt	ncs	gag	; cat	: tct	gct	ccc	ata	41	gtt	cont	t cs	11 a.:	LCI.	CAA	288
Xaa	XBA	Сув	X.e.a	Gli	H11	941	Ala	Pro	Ila	Lys	y Val	. Хав	ı Xa	a Xa	ta 1	Gla	•••
				85					90						95		
														-	-		
acg	ggt	aan	aag	tac	: con	tgg	gat	gca	gaa	act	caa	994	. tgr	a et	it i	ete	336
Thr	Gly	Xaa	Lys	Tyr	X.a.e	Tip	Asp	Ala	G} <i>⁄</i> π	Thr	Gln	Gly	Tr	o Il	e l	Len	
			100					105				_	11				
dan	ten	ett	ttn	tat	930	tac	atc	8t¢	ACA	can	att	cct	ggt	99	a (tat	384
Xaa	Xex	Phe	Xaa	Tyr	Gly	Tyr	Ile	I1e	Thr	Xaa	Ile	Pro	G1 ₃	gi	y i	Tyz-	
		115					120					125			-	_	
gtt	gcc	Bgc	ana	AID	333	339	een	ntg	ntg	cta	gga	ttn	399	, at	c r	itt	432
Val		Ser	Xaa	Kaa	Gly	Gly	Xaa	XB A	XAL	Leu	Gly	Xaa	Gly	, II	e 2	aa	
	130					135				•	160						
gnn	acn	gct	ntc	ntc	AEC	ctg	ttc	act	ccc	ntn	gct	gea	gat	tt	n g	ga	480
	ī,a a	Ala	XRA	Xaa	Thr	Leu	Phe	Thr	Pro	Xaa	Ala	Ala	æp	Xa	a, G	1y	
145					150				•	155					1	60	
gtn	33 8	nçn	ota	ntt	gna	ctc	egn (gca ·	cta	gaa	āār	eta	3 3a	gag	3 9	gt	528
Xaa	Θlγ	XAA	X#A	AAK	Xaa	Leu	xaa :	Ala :	Leu	۵ì a	Xaa	Leu	Gly	Gli	ı G	ly	
				165					170					175		-	
					-												
âţ'n	aca	tnt	CCA	âcc	atg	cat	acc 1	atg	gg	tct	ten	tgg	gct	CC(: ¢	c t	576
Xaa	Thr	Xaa	Pro	Ala	Ket	Hie .	Ala I	det :	Çzp	ŝer	Xaa	Trp	Ala	Pro	P	ro	
			180					LAS				_	190				

							Ile					Ala	ctt Leu	624
						Leu							atg Met	672
													tgg Trp 240	720
						gtt Val							_	768
				_	_	gan Xaa							aat Asn	\$16
_			_	_		gtg Val 280	_							B64
						gtt Val								912
			_			ttg Lau				_	-	_		960
						a 1y 999							Lou	1008
			_	_		Lo u Ctg					_	•		1056
	-					act Thr 360		_	-	XAB			-	1104
				aly		gen Xaa			Leu					1152

		_	•		ten	_	_	_	_							2200
BEX	Gly	СуБ	АБР	Tyr	X a a	Leu	ABX	Val	Xaa	Phe	Leu	Хва	IJè	Ser	Thr	
385					390					395					400	
асл	etg	gga	āāc	ttt	tgc	tet	tet	gga	ttt	agc	atc	ae¢	cat	ctg	gan	1248
Xaa	Lau	Gly	GJA	Phe	Сув	Ser	Ser	Gly	Phe	Ser	Ile	Asn	His	leu	Xaa	
			•	405					410					415		
att	gct	cct	tcg	tet	ģct	ggt	atn	ctc	ctg	gg¢	atc	aca	aat	acn	ttt	1296
Ile	Ala	Pro	Ser	Tyr	Ala	Gly	Xaa	Leu	Leu	Gly	Ile	Thr	Asn	Xaa	Phe	
			420					425					430			
gcc	act	att	cen	gga	atg	ntt	999	CCC	πtc	att	ĝen	ana	agt	ctn	acc	1344
Ala	The	Ile	Xaa	Gly	MeŁ	AAX	Qly	Pro	Xaa	Ile	Xaa	BAK	Ser	Xaa	Thr	
		435					440					445				
cct	gan	aac	act	ntt	gga	gea	tgg	caa	açm	gtn	ttc	tnn	atn	ģct	get	1392
Pro	Xza	Авп	Thr	Xaa	Gly	Glu	Trp	Gln	Xaa	Xaa	Phe	XBB	Xaa	Ala	Ala	
	450					455					460					
gct	atn	aat	ģtn	ttt	gg t	gco	att	ttc	ttn	aça	cta	ttc	gcc	384	ggt	1440
Ala	XBS	Asn	BaX	Phe	Gly	Mic	Ile	Phe	Xaa	Thr	Lau	Phe	Ala	Lys	Gly	
465					470					475					480	
•	-				gen								_			1485
Gl u	Xaa	Gln	Asi	-	XAA	Xaa	Xea	Asp		XAB	GIA	RTO	Arg			
				485					490					495		

<210> 12

<211> 495

<212> PRT

<213> Artificial Bequence

<400> 12

Met Xaa Xaa Pro Val Xaa Asp Kaa Ala Xaa Xaa Gly Glu Glu Xaa

Kaa Asp Arg Xaa Xaa Xaa Xaa Xaa Ala Xaa Arg Xaa Glu Xaa Ala 20 25 30

Pro Xea Cys Cys Ser Ala Arg Tyr Asn Xaa Ala Xea Leu Xaa Phe Phe 35 40 45

Gly Phe Phe Xaa Xaa Tyr Xaa Leu Xaa Val Asn Leu Xaa Val Xaa Xaa 50 55 60

Val Xoa Met Xaa Asp Ser Xas Thr Thr Xaa Xaa Asp Asn Arg Xaa Ser

-	

70

75

80

Xax Xaa Cys Xaa Glu His Ser Ala Pro Ile Lys Val Xaa Xaa Xaa Gln 85 90 95

Thr Gly Xaa Lys Tyr Xaa Trp Asp Ala Glu Thr Gln Gly Trp Ile Leu 100 105 110

Xaa Xaa Phe Xaa Tyr Gly Tyr Ile Ile Thr Xaa Ile Pro Gly Gly Tyr
115 120 125

Val Ala Ser Xaa Xaa Gly Gly Xaa Xaa Xaa Leu Gly Xaa Gly 11e Xaa 130 135 140

Xaa Xaa Ala Xaa Xaa Thr Leu Phe Thr Pro Xaa Ala Ala Asp Xaa Gly
145 150 155 150

Xaa Gly Xaa Xaa Xaa Xaa Leu Xaa Ala Leu Glu Xaa Leu Gly Glu Gly 165 170 175

Xaa Thr Xaa Pro Ala Met His Ala Met Trp Ser Kaa Trp Ala Pro Pro 180 185 190

Leu Glu Arg Ser Xaa Leu Xaa Xaa Ile Xaa Tyr Ala Gly Ala Xaa Leu 195 200 205

Gly Thr Val Xaa Ser Leu Pro Leu Ser Gly Xaa Ile Cys Tyr Tyr Met 210 215 220

Asn Trp Thr Tyr Val Phe Xas Xas Phe Xas Xas Xas Gly Xas Xas Trp 225 230 235 240

Phe Xas Xss Trp Ile Xas Leu Val Ser Xas Thr Pro Xas Xas His Lys 245 250 255

Xae Xaa Xaa Tyr Glu Lys Xaa Xas Ile Leu Ser Ser Leu Xaa Asn 260 265 270

Gin Kaa Ser Ser Gin Lys Ser Vel Pro Trp Kaa Kaa Kaa Kaa Lys Xaa 275 280 285

Leu Pro Leu Trp Ala Xaa Xaa Val Ala Xaa Phe Ser Tyr Asn Trp Thr 290 295 300

Phe Tyr Thr Xae Leu Xaa Leu Leu Pro Thr Xaa Met Lys Xaa Xam Leu 305 310 315 320

Arg Phe Asn Xea Gln Glu Asn Gly Phe Lau Ser Xea Xea Pro Tyr Leu

7	7	=
_3	4	2

33	D
----	---

335

- Xaa Xaa Trp Leu Cys Met 11e Leu Xaa Gly Gln Ala Ala Asp Asn Leu 340 345 350
- Arg Ala Xaa Tro Aan Phe Ser Thr Xaa Xaa Val Xaa Arg Xaa Phe Ser 355 360 365
- Leu Ile Xaa Met Ile Gly Pro Xaa Xaa Phe Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa 370 380
- Xas Gly Cys Asp Tyr Xsa Leu Xas Val Xsa Phe Leu Xsa Ile Ser Thr 385 390 395 400
- Xaa Lau Gly Gly Phe Cya Ser Ser Gly Phe Ser Ila Asn His Lau Xaa 405 410 415
- Ile Ala Pro Ser Tyr Ala Gly Xas Leu Leu Gly Ile Thr Asn Xaa Phe 420 425 430
- Ala Thr Ile XBB Gly Met XBB Gly Pro XBB Ile XBB XBB Ser XBB Thr
 435 440 445
- Pro Xea Asn Thr Xea Gly Glu Trp Gln Xea Zee Phe Xea Zee Ala Ala 450 455 460
- Ala Xaa Asn Xaa Phe Gly Ala Ile Phe Xaa Thr Leu Phe Ala Lys Gly
 465 470 475 480
- Glu Xaa Gln Asn Trp Xaa Xaa Xaa Asp His Xaa Gly His Ary Xaa 485 490 495